AO 120 (Rev. 3/04)

TO:	REPORT ON THE FILING OR DETERMINATION OF AN ACTION REGARDING A PATENT OR TRADEMARK
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In Compliance with 35 U.S.C. § 290 and/or 15 U.S.C. § 1116 you are hereby advised that a court action has been filed in the U.S. District Court Eastern District of New York on the following X Patents or ☐ Trademarks:

DOCKET NO.	DATE FILED	U.S. DISTRICT COURT
CV 10-661-ADS-AKT	2/16/2010	EDNY, 100 Federal Plaza, P.O. Box 9014, Central Islip, NY 11722

PLAINTIFF	DEFENDANT
Cold Spring Harbor Laboratory	Ropes & Gray LLP Matthew P. Vincent

PATENT OR TRADEMARK NO.	DATE OF PATENT OR TRADEMARK	HOLDER OF PATENT OR TRADEMARK
1 6,506,559		**See attached complaint**
2		
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In the above—entitled case, the following patent(s)/ trademark(s) have been included:

DATE INCLUDED

INCLUDED BY ☐ Amendment ☐ Answer ☐ Cross Bill ☐ Other Pleading

PATENT OR TRADEMARK NO.	DATE OF PATENT OR TRADEMARK	HOLDER OF PATENT OR TRADEMARK
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In the above—entitled case, the following decision has been rendered or judgement issued:

DECISION/JUDGEMENT

CLERK	(BY) DEPUTY CLERK	DATE
ROBERT C. HEINEMANN		

Copy 1—Upon initiation of action, mail this copy to Director Copy 3—Upon termination of action, mail this copy to Director
Copy 2—Upon filing document adding patent(s), mail this copy to Director Copy 4—Case file copy

25. Despite the fact that Vincent was fully aware of the Fire application as early as October 2000, when he first copied text from that application into the '097 application, R&G did not cite the Fire application in any papers filed on behalf of CSHL until the Supplemental Information Disclosure Statement of November 26, 2004. And, R&G did not cite the Fire Patent in any papers filed on behalf of CSHL until the Supplemental Information Disclosure Statement of January 7, 2005.

26. The '435 PCT application, filed March 16, 2001, the '862 application filed May 16, 2001, and the '557 application filed May 24, 2001 are directed generally to the initial methods and technologies Dr. Hannon developed relating to use of RNA interference in mammalian and other cells, including use of hairpin RNAs to regulate target genes. In particular, the filed '557 application included claims (20-25) directed to use of hairpin RNAs to inhibit gene expression and expression of such hairpin RNAs in cells of a transgenic non-human mammal.

27. About one half of the "Detailed Description of Certain Preferred Embodiments" (hereinafter, the "Detailed Description") found in the three earliest filed non-provisional Hannon Applications consists of text copied from the Fire application. Vincent's failure to provide an adequate description of Dr. Hannon's technology in these applications seriously compromised the ability of these applications, in particular the '557 application, to serve as priority support for Dr. Hannon's patent claims. This fact has deprived CSHL of the opportunity to obtain allowance of claims covering Dr. Hannon's inventions entitled to the respective filing dates of these applications, based on the support from these applications.

28. Vincent repeatedly effectuated this copying of Fire, notwithstanding that he knew from the outset that the Hannon Applications needed to be distinguished from Fire.

Argonaut gene. For instance, the gene activation construct can replace the endogenous promoter of a Dicer or Argonaut gene with a heterologous promoter, e.g., one which causes constitutive expression of the Dicer or Argonaut gene or which causes inducible expression of the gene under conditions different from the normal expression pattern of Dicer or Argonaut. A variety of different formats for the gene activation constructs are available. See, for example, the Transkaryotic Therapies, Inc PCT publications WO93/09222, WO95/31560, WO96/29411, WO95/31560 and WO94/12650.

[0112] In preferred embodiments, the nucleotide sequence used as the gene activation construct can be comprised of (1) DNA from some portion of the endogenous Dicer or Argonaut gene (exon sequence, intron sequence, promoter sequences, etc.) which direct recombination and (2) heterologous transcriptional regulatory sequence(s) which is to be operably linked to the coding sequence for the genomic Dicer or Argonaut gene upon recombination of the gene activation construct. For use in generating cultures of Dicer or Argonaut producing cells, the construct may further include a reporter gene to detect the presence of the knockout construct in the cell.

[0113] The gene activation construct is inserted into a cell, and integrates with the genomic DNA of the cell in such a position so as to provide the heterologous regulatory sequences in operative association with the native Dicer or Argonaut gene. Such insertion occurs by homologous recombination, i.e., recombination regions of the activation construct that are homologous to the endogenous Dicer or Argonaut gene sequence hybridize to the genomic DNA and recombine with the genomic sequences so that the construct is incorporated into the corresponding position of the genomic DNA.

[0114] The terms "recombination region" or "targeting sequence" refer to a segment (i.e., a portion) of a gene activation construct having a sequence that is substantially identical to or substantially complementary to a genomic gene sequence, e.g., including 5' flanking sequences of the genomic gene, and can facilitate homologous recombination between the genomic sequence and the targeting transgene construct.

[0115] As used herein, the term "replacement region" refers to a portion of a activation construct which becomes integrated into an endogenous chromosomal location following homologous recombination between a recombination region and a genomic sequence.

[0116] The heterologous regulatory sequences, e.g., which are provided in the replacement region, can include one or more of a variety elements, including: promoters (such as constitutive or inducible promoters), enhancers, negative regulatory elements, locus control regions, transcription factor binding sites, or combinations thereof.

[0117] Promoters/enhancers which may be used to control expression of the targeted gene in vivo include, but are not limited to, the cytomegalovirus (CMV) promoter/enhancer (Karasuyama et al., 1989, *J. Exp. Med.*, 169:13), the human β-actin promoter (Gunning et al. (1987) *PNAS* 84:4831-4835), the glucocorticoid-inducible promoter present in the mouse mammary tumor virus long terminal repeat (MMTV LTR) (Klessig et al. (1984) *Mol. Cell Biol.* 4:1354-1362), the long terminal repeat sequences of Moloney murine leukemia virus (MuLV LTR) (Weiss et al. (1985) *RNA Tumor Viruses*, Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.), the SV40 early or late region promoter (Bernoist et al. (1981) *Nature* 290:304-310; Templeton et al. (1984) *Mol. Cell Biol.*, 4:817; and Sprague et al. (1983) *J. Virol.*, 45:773), the promoter contained in the 3' long terminal repeat of Rous sarcoma virus (RSV) (Yamamoto et al., 1980, *Cell*, 22:787-797), the herpes simplex virus (HSV) thymidine kinase promoter/enhancer (Wagner et al. (1981) *PNAS* 82:3567-71), and the herpes simplex virus LAT promoter (Wolfe et al. (1992) *Nature Genetics*, 1:379-384).

[0118] In still other embodiments, the replacement region merely deletes a negative transcriptional control element of the native gene, e.g., to activate expression, or ablates a positive control element, e.g., to inhibit expression of the targeted gene.

[0119] B. Cell/Organism

[0120] The cell with the target gene may be derived from or contained in any organism (e.g., plant, animal, protozoan, virus, bacterium, or fungus). The dsRNA construct may be synthesized either in vivo or in vitro. Endogenous RNA polymerase of the cell may mediate transcription in vivo, or cloned RNA polymerase can be used for transcription in vivo or in vitro. For generating double stranded transcripts from a transgene in vivo, a regulatory region may be used to transcribe the RNA strand (or strands).

[0121] Furthermore, genetic manipulation becomes possible in organisms that are not classical genetic models. Breeding and screening programs may be accelerated by the ability to rapidly assay the consequences of a specific, targeted gene disruption. Gene disruptions may be used to discover the function of the target gene, to produce disease models in which the target gene are involved in causing or preventing a pathological condition, and to produce organisms with improved economic properties.

[0122] The cell with the target gene may be derived from or contained in any organism. The organism may a plant, animal, protozoan, bacterium, virus, or fungus. The plant may be a monocot, dicot or gymnosperm; the animal may be a vertebrate or invertebrate. Preferred microbes are those used in agriculture or by industry, and those that are pathogenic for plants or animals. Fungi include organisms in both the mold and yeast morphologies.

[0123] Plants include arabidopsis; field crops (e.g., alfalfa, barley, bean, corn, cotton, flax, pea, rape, rice, rye, safflower, sorghum, soybean, sunflower, tobacco, and wheat); vegetable crops (e.g., asparagus, beet, broccoli, cabbage, carrot, cauliflower, celery, cucumber, eggplant, lettuce, onion, pepper, potato, pumpkin, radish, spinach, squash, taro, tomato, and zucchini); fruit and nut crops (e.g., almond, apple, apricot, banana, blackberry, blueberry, cacao, cherry, coconut, cranberry, date, faJoa, filbert, grape, grapefruit, guava, kiwi, lemon, lime, mango, melon, nectarine, orange, papaya, passion fruit, peach, peanut, pear, pineapple, pistachio, plum, raspberry, strawberry, tangerine, walnut, and watermelon); and ornamentals (e.g., alder, ash, aspen, azalea, birch, boxwood, camellia, carnation, chrysanthemum, elm, fir, ivy, jasmine, juniper, oak, palm, poplar, pine, redwood, rhododendron, rose, and rubber).

[0124] Examples of vertebrate animals include fish, mammal, cattle, goat, pig, sheep, rodent, hamster, mouse, rat, primate, and human.

[0125] Invertebrate animals include nematodes, other worms, drosophila, and other insects. Representative generae of nematodes include those that infect animals (e.g., Ancylostoma, Ascaridia, Ascaris, Bunostomum, Caenorhabditis, Capillaria, Chabertia, Cooperia, Dictyocaulus, Haemonchus, Heterakis, Nematodirus, Oesophagostomum, Ostertagia, Oxyuris, Parascaris, Strongylus, Toxascaris, Trichuris, Trichostrongylus, Tflichonema, Toxocara, Uncinaria) and those that infect plants (e.g., *B ursaphalenchus*, Criconerriella, Diiylenchus, Ditylenchus, Globodera, Helicotylenchus, Heterodera, Longidorus, Melodoigyne, Nacobbus, Paratylenchus, Pratylenchus, Radopholus, Rotelynchus, Tylenchus, and Xiphinerna). Representative orders of insects include Coleoptera, Diptera, Lepidoptera, and Homoptera.

[0126] The cell having the target gene may be from the germ line or somatic, totipotent or pluripotent, dividing or non-dividing, parenchyma or epithelium, immortalized or transformed, or the like. The cell may be a stem cell or a differentiated cell. Cell types that are differentiated include adipocytes, fibroblasts, myocytes, cardiomyocytes, endothelium, neurons, glia, blood cells, megakaryocytes, lymphocytes, macrophages, neutrophils, eosinophils, basophils, mast cells, leukocytes, granulocytes, keratinocytes, chondrocytes, osteoblasts, osteoclasts, hepatocytes, and cells of the endocrine or exocrine glands.

[0127] C. Targeted Genes

[0128] The target gene may be a gene derived from the cell, an endogenous gene, a transgene, or a gene of a pathogen which is present in the cell after infection thereof. Depending on the particular target gene and the dose of double stranded RNA material delivered, the procedure may provide partial or complete loss of function for the target gene. Lower doses of injected material and longer times after administration of dsRNA may result in inhibition in a smaller fraction of cells. Quantitation of gene expression in a cell may show similar amounts of inhibition at the level of accumulation of target mRNA or translation of target protein.

[0129] "Inhibition of gene expression" refers to the absence (or observable decrease) in the level of protein and/or mRNA product from a target gene. "Specificity" refers to the ability to inhibit the target gene without manifest effects on other genes of the cell. The consequences of inhibition can be confirmed by examination of the outward properties of the cell or organism (as presented below in the examples) or by biochemical techniques such as RNA solution hybridization, nuclease protection, Northern hybridization, reverse transcription, gene expression monitoring with a microarray, antibody binding, enzyme linked immunosorbent assay (ELISA), Western blotting, radioImmunoassay (RIA), other immunoassays, and fluorescence activated cell analysis (FACS). For RNA-mediated inhibition in a cell line or whole organism, gene expression is conveniently assayed by use of a reporter or drug resistance gene whose protein product is easily assayed. Such reporter genes include acetohydroxyacid synthase (AHAS), alkaline phosphatase (AP), beta galactosidase (LacZ), beta glucoronidase (GUS), chloramphenicol acetyltransferase (CAT), green fluorescent protein (GFP), horseradish peroxidase (HRP), luciferase (Luc), nopaline synthase (NOS), octopine synthase (OCS), and derivatives thereof multiple selectable markers are available that confer resistance to ampicillin, bleomycin, chloramphenicol, gentamycin, hygromycin, kanamycin, lincomycin, methotrexate, phosphinothricin, puromycin, and tetracyclin.

[0130] Depending on the assay, quantitation of the amount of gene expression allows one to determine a degree of inhibition which is greater than 10%, 33%, 50%, 90%, 95% or 99% as compared to a cell not treated according to the present invention. Lower doses of injected material and longer times after administration of dsRNA may result in inhibition in a smaller fraction of cells (e.g., at least 10%, 20%, 50%, 75%,90%, or 95% of targeted cells). Quantitation of gene expression in a cell may show similar amounts of inhibition at the level of accumulation of target mRNA or translation of target protein. As an example, the efficiency of inhibition may be determined by assessing the amount of gene product in the cell: mRNA may be detected with a hybridization probe having a nucleotide sequence outside the region used for the inhibitory double-stranded RNA, or translated polypeptide may be detected with an antibody raised against the polypeptide sequence of that region.

[0131] As disclosed herein, the present invention may is not limited to any type of target gene or nucleotide sequence. But the following classes of possible target genes are listed for illustrative purposes: developmental genes (e.g., adhesion molecules, cyclin kinase inhibitors, Writ family members, Pax family members, Winged helix family members, Hox family members, cytokines/lymphokines and their receptors, growth/differentiation factors and their receptors, neurotransmitters and their receptors); oncogenes (e.g., ABLI, BCLI, BCL2, BCL6, CBFA2, CBL, CSFIR, ERBA, ERBB, EBRB2, ETSI, ETS1, ETV6, FGR, FOS, FYN, HCR, HRAS, JUN, KRAS, LCK, LYN, MDM2, MLL, MYB, MYC, MYCLI, MYCN, NRAS, PIM 1, PML, RET, SRC, TALI, TCL3, and YES); tumor suppressor genes (e.g., APC, BRCA 1, BRCA2, MADH4, MCC, NF 1, NF2, RB 1, TP53, and WTI); and enzymes (e.g., ACC synthases and oxidases, ACP desaturases and hydroxylases, ADP-glucose pyrophorylases, ATPases, alcohol dehydrogenases, amylases, amyloglucosidases, catalases, cellulases, chalcone synthases, chitinases, cyclooxygenases, decarboxylases, dextrinases, DNA and RNA polymerases, galactosidases, glucanases, glucose oxidases, granule-bound starch synthases, GTPases, helicases, hemicellulases, integrases, inulinases, invertases, isomerases, kinases, lactases, lipases, lipoxygenases, lysozymes, nopaline synthases, octopine synthases, pectinesterases, peroxidases, phosphatases, phospholipases, phosphorylases, phytases, plant growth regulator synthases, polygalacturonases, proteinases and peptidases, pullanases, recombinases, reverse transcriptases, RUBISCOs, topoisomerases, and xylanases).

[0132] D. dsRNA constructs

[0133] The dsRNA construct may comprise one or more strands of polymerized ribonucleotide. It may include modifications to either the phosphate-sugar backbone or the nucleoside. For example, the phosphodiester linkages of natural RNA may be modified to include at least one of a nitrogen or sulfur heteroatom. Modifications in RNA structure may be tailored to allow specific genetic inhibition

while avoiding a general panic response in some organisms which is generated by dsRNA. Likewise, bases may be modified to block the activity of adenosine deaminase. The dsRNA construct may be produced enzymatically or by partial/total organic synthesis, any modified ribonucleotide can be introduced by in vitro enzymatic or organic synthesis.

[0134] The dsRNA construct may be directly introduced into the cell (i.e., intracellularly); or introduced extracellularly into a cavity, interstitial space, into the circulation of an organism, introduced orally, or may be introduced by bathing an organism in a solution containing RNA. Methods for oral introduction include direct mixing of RNA with food of the organism, as well as engineered approaches in which a species that is used as food is engineered to express an RNA, then fed to the organism to be affected. Physical methods of introducing nucleic, acids include injection directly into the cell or extracellular injection into the organism of an RNA solution.

[0135] The double-stranded structure may be formed by a single self-complementary RNA strand or two complementary RNA strands. RNA duplex formation may be initiated either inside or outside the cell. The RNA may be introduced in an amount which allows delivery of at least one copy per cell. Higher doses (e.g., at least 5, 10, 100, 500 or 1000 copies per cell) of double-stranded material may yield more effective inhibition; lower doses may also be useful for specific applications. Inhibition is sequence-specific in that nucleotide sequences corresponding to the duplex region of the RNA are targeted for genetic inhibition.

[0136] dsRNA constructs containing a nucleotide sequences identical to a portion of the target gene are preferred for inhibition. RNA sequences with insertions, deletions, and single point mutations relative to the target sequence have also been found to be effective for inhibition. Thus, sequence identity may optimized by sequence comparison and alignment algorithms known in the art (see Gribskov and Devereux, Sequence Analysis Primer, Stockton Press, 1991, and references cited therein) and calculating the percent difference between the nucleotide sequences by, for example, the Smith-Waterman algorithm as implemented in the BESTFIT software program using default parameters (e.g., University of Wisconsin Genetic Computing Group). Greater than 90% sequence identity, or even 100% sequence identity, between the inhibitory RNA and the portion of the target gene is preferred. Alternatively, the duplex region of the RNA may be defined functionally as a nucleotide sequence that is capable of hybridizing with a portion of the target gene transcript (e.g., 400 mM NaCl, 40 mM PIPES pH 6.4, 1 mM EDTA, 50° C. or 70° C. hybridization for 12-16 hours; followed by washing). In certain preferred embodiments, the length of the dsRNA is at least 20, 21 or 22 nucleotides in length, e.g., corresponding in size to RNA products produced by Dicer-dependent cleavage. In certain embodiments, the dsRNA construct is at least 25, 50, 100, 200, 300 or 400 bases. In certain embodiments, the dsRNA construct is 400-800 bases in length.

[0137] 100% sequence identity between the RNA and the target gene is not required to practice the present invention. Thus the invention has the advantage of being able to tolerate sequence variations that might be expected due to genetic mutation, strain polymorphism, or evolutionary divergence.

[0138] The dsRNA construct may be synthesized either in vivo or in vitro. Endogenous RNA polymerase of the cell may mediate transcription in vivo, or cloned RNA polymerase can be used for transcription in vivo or in vitro. For transcription from a transgene in vivo or an expression construct, a regulatory region (e.g., promoter, enhancer, silencer, splice donor and acceptor, polyadenylation) may be used to transcribe the dsRNA strand (or strands). Inhibition may be targeted by specific transcription in an organ, tissue, or cell type; stimulation of an environmental condition (e.g., infection, stress, temperature, chemical inducers); and/or engineering transcription at a developmental stage or age. The RNA strands may or may not be polyadenylated; the RNA strands may or may not be capable of being translated into a polypeptide by a cell's translational apparatus. The dsRNA construct may be chemically or enzymatically synthesized by manual or automated reactions. The dsRNA construct may be synthesized by a cellular RNA polymerase or a bacteriophage RNA polymerase (e.g., T3, T7, SP6). The use and production of an expression construct are known in the art 32,33,34 (see also WO 97/32016; U.S. Pat. Nos. 5,593,874, 5,698,425, 5,712,135, 5,789,214, and 5,804,693; and the references cited therein). If synthesized chemically or by in vitro enzymatic synthesis, the RNA may be purified prior to introduction into the cell. For example, RNA can be purified from a mixture by extraction with a solvent or resin, precipitation, electrophoresis, chromatography or a combination thereof. Alternatively, the dsRNA construct may be used with no or a minimum of purification to avoid losses due to sample processing. The dsRNA construct may be dried for storage or dissolved in an aqueous solution. The solution may contain buffers or salts to promote annealing, and/or stabilization of the duplex strands.

[0139] Physical methods of introducing nucleic acids include injection of a solution containing the dsRNA construct, bombardment by particles covered by the dsRNA construct, soaking the cell or organism in a solution of the RNA, or electroporation of cell membranes in the presence of the dsRNA construct. A viral construct packaged into a viral particle would accomplish both efficient introduction of an expression construct into the cell and transcription of dsRNA construct encoded by the expression construct. Other methods known in the art for introducing nucleic acids to cells may be used, such as lipid-mediated carrier transport, chemicalmediated transport, such as calcium phosphate, and the like. Thus the dsRNA construct may be introduced along with components that perform one or more of the following activities: enhance RNA uptake by the cell, promote annealing of the duplex strands, stabilize the annealed strands, or other-wise increase inhibition of the target gene.

[0140] E. Illustrative Uses

[0141] One utility of the present invention is as a method of identifying gene function in an organism, especially higher eukaryotes comprising the use of double-stranded RNA to inhibit the activity of a target gene of previously unknown function. Instead of the time consuming and laborious isolation of mutants by traditional genetic screening, functional genomics would envision determining the function of uncharacterized genes by employing the invention to reduce the amount and/or alter the timing of target gene activity. The invention could be used in determining potential targets for pharmaceutics, understanding normal

and pathological events associated with development, determining signaling pathways responsible for postnatal development/aging, and the like. The increasing speed of acquiring nucleotide sequence information from genomic and expressed gene sources, including total sequences for mammalian genomes, can be coupled with the invention to determine gene function in a cell or in a whole organism. The preference of different organisms to use particular codons, searching sequence databases for related gene products, correlating the linkage map of genetic traits with the physical map from which the nucleotide sequences are derived, and artificial intelligence methods may be used to define putative open reading frames from the nucleotide sequences acquired in such sequencing projects.

[0142] A simple assay would be to inhibit gene expression according to the partial sequence available from an expressed sequence tag (EST). Functional alterations in growth, development, metabolism, disease resistance, or other biological processes would be indicative of the normal role of the EST's gene product.

[0143] The ease with which the dsRNA construct can be introduced into an intact cell/organism containing the target gene allows the present invention to be used in high throughput screening (HTS). For example, duplex RNA can be produced by an amplification reaction using primers flanking the inserts of any gene library derived from the target cell or organism. Inserts may be derived from genomic DNA or mRNA (e.g., cDNA and cRNA). Individual clones from the library can be replicated and then isolated in separate reactions, but preferably the library is maintained in individual reaction vessels (e.g., a 96 well microtiter plate) to minimize the number of steps required to practice the invention and to allow automation of the process.

[0144] In an exemplary embodiment, the subject invention provides an arrayed library of RNAi constructs. The array may in the form of solutions, such as multi-well plates, or may be "printed" on solid substrates upon which cells can be grown. To illustrate, solutions containing duplex RNAs that are capable of inhibiting the different expressed genes can be placed into individual wells positioned on a microtiter plate as an ordered array, and intact cells/organisms in each well can be assayed for any changes or modifications in behavior or development due to inhibition of target gene activity.

[0145] In one embodiment, the subject method uses an arrayed library of RNAi constructs to screen for combinations of RNAi that is lethal to host cells. Synthetic lethality is a bedrock principle of experimental genetics. A synthetic lethality describes the properties of two mutations which, individually, are tolerated by the organism but which, in combination, are lethal. The subject arrays can be used to identify loss-of-function mutations that are lethal in combination with alterations in other genes, such as activated oncogenes or loss-of-function mutations to tumor suppressors. To achieve this, one can create "phenotype arrays" using cultured cells. Expression of each of a set of genes, such as the host cell's genome, can be individually systematically disrupted using RNA interference. Combination with alterations in oncogene and tumor suppressor pathways can be used to identify synthetic lethal interactions that may identify novel therapeutic targets.

[0146] In certain embodiments, the RNAi constructs can be fed directly to, injected into, the cell/organism containing the target gene. Alternatively, the duplex RNA can be produced by in vivo or in vitro transcription from an expression construct used to produce the library. The construct can be replicated as individual clones of the library and transcribed to produce the RNA; each clone can then be fed to, or injected into, the cell/organism containing the target gene. The function of the target gene can be assayed from the effects it has on the cell/organism when gene activity is inhibited. This screening could be amenable to small subjects that can be processed in large number, for example, tissue culture cells derived from mammals, especially primates, and most preferably humans.

[0147] If a characteristic of an organism is determined to be genetically linked to a polymorphism through RFLP or QTL analysis, the present invention can be used to gain insight regarding whether that genetic polymorphism might be directly responsible for the characteristic. For example, a fragment defining the genetic polymorphism or sequences in the vicinity of such a genetic polymorphism can be amplified to produce an RNA, the duplex RNA can be introduced to the organism or cell, and whether an alteration in the characteristic is correlated with inhibition can be determined. Of course, there may be trivial explanations for negative results with this type of assay, for example: inhibition of the target gene causes lethality, inhibition of the target gene may not result in any observable alteration, the fragment contains nucleotide sequences that are not capable of inhibiting the target gene, or the target gene's activity is redundant.

[0148] The present invention may be useful in allowing the inhibition of essential genes. Such genes may be required for cell or organism viability at only particular stages of development or cellular compartments. The functional equivalent of conditional mutations may be produced by inhibiting activity of the target gene when or where it is not required for viability. The invention allows addition of RNA at specific times of development and locations in the organism without introducing permanent mutations into the target genome.

[0149] If alternative splicing produced a family of transcripts that were distinguished by usage of characteristic exons, the present invention can target inhibition through the appropriate exons to specifically inhibit or to distinguish among the functions of family members. For example, a hormone that contained an alternatively spliced transmembrane domain may be expressed in both membrane bound and secreted forms. Instead of isolating a nonsense mutation that terminates translation before the transmembrane domain, the functional consequences of having only secreted hormone can be determined according to the invention by targeting the exon containing the transmembrane domain and thereby inhibiting expression of membrane-bound hormone.

[0150] The present invention may be used alone or as a component of a kit having at least one of the reagents necessary to carry out the in vitro or in vivo introduction of RNA to test samples or subjects. Preferred components are the dsRNA and a vehicle that promotes introduction of the dsRNA. Such a kit may also include instructions to allow a user of the kit to practice the invention.

[0151] Alternatively, an organism may be engineered to produce dsRNA which produces commercially or medically

beneficial results, for example, resistance to a pathogen or its pathogenic effects, improved growth, or novel developmental patterns.

IV. Exemplification

[0152] The invention, now being generally described, will be more readily understood by reference to the following examples, which are included merely for purposes of illustration of certain aspects and embodiments of the present invention and are not intended to limit the invention.

Example 1

An RNA-directed Nuclease Mediates RNAi Gene Silencing

[0153] In a diverse group of organisms that includes *Caenorhabditis elegans*, Drosophila, planaria, hydra, trypanosomes, fungi and plants, the introduction of double-stranded RNAs inhibits gene expression in a sequence-specific manner[1-7]. These responses, called RNA interference or post-transcriptional gene silencing, may provide anti-viral defence, modulate transposition or regulate gene expression[1, 6, 8-10]. We have taken a biochemical approach towards elucidating the mechanisms underlying this genetic phenomenon. Here we show that 'loss-of-function' phenotypes can be created in cultured Drosophila cells by transfection with specific double-stranded RNAs. This coincides with a marked reduction in the level of cognate cellular messenger RNAs. Extracts of transfected cells contain a nuclease activity that specifically degrades exogenous transcripts homologous to transfected double-stranded RNA. This enzyme contains an essential RNA component. After partial purification, the sequence-specific nuclease co-fractionates with a discrete, ~25-nucleotide RNA species which may confer specificity to the enzyme through homology to the substrate mRNAs.

[0154] Although double-stranded RNAs (dsRNAs) can provoke gene silencing in numerous biological contexts including Drosophila[11, 12], the mechanisms underlying this phenomenon have remained mostly unknown. We therefore wanted to establish a biochemically tractable model in which such mechanisms could be investigated.

[0155] Transient transfection of cultured, Drosophila S2 cells with a lacZ expression vector resulted in β-galactosidase activity that was easily detectable by an in situ assay (**FIG. 1***a*). This activity was greatly reduced by co-transfection with a dsRNA corresponding to the first 300 nucleotides of the lacZ sequence, whereas co-transfection with a control dsRNA (CD8) (**FIG. 1***a*) or with single-stranded RNAs of either sense or antisense orientation (data not shown) had little or no effect. This indicated that dsRNAs could interfere, in a sequence-specific fashion, with gene expression in cultured cells.

[0156] To determine whether RNA interference (RNAi) could be used to target endogenous genes, we transfected S2 cells with a dsRNA corresponding to the first 540 nucleotides of Drosophila cyclin E, a gene that is essential for progression into S phase of the cell cycle. During log-phase growth, untreated S2 cells reside primarily in G2/M (**FIG. 1***b*). Transfection with lacZ dsRNA had no effect on cell-cycle distribution, but transfection with the cyclin E dsRNA caused a G1-phase cell-cycle arrest (**FIG. 1***b*). The ability of cyclin E dsRNA to provoke this response was length-dependent. Double-stranded RNAs of 540 and 400 nucleotides were quite effective, whereas dsRNAs of 200 and 300 nucleotides were less potent. Double-stranded cyclin E RNAs of 50 or 100 nucleotides were inert in our assay, and transfection with a single-stranded, antisense cyclin E RNA had virtually no effect.

[0157] One hallmark of RNAi is a reduction in the level of mRNAs that are homologous to the dsRNA. Cells transfected with the cyclin E dsRNA (bulk population) showed diminished endogenous cyclin E mRNA as compared with control cells (**FIG. 1***c*). Similarly, transfection of cells with dsRNAs homologous to fizzy, a component of the anaphase-promoting complex (APC) or cyclin A, a cyclin that acts in S, G2 and M, also caused reduction of their cognate mRNAs (**FIG. 1***c*). The modest reduction in fizzy mRNA levels in cells transfected with cyclin A dsRNA probably resulted from arrest at a point in the division cycle at which fizzy transcription is low[14, 15]. These results indicate that RNAi may be a generally applicable method for probing gene function in cultured Drosophila cells.

[0158] The decrease in mRNA levels observed upon transfection of specific dsRNAs into Drosophila cells could be explained by effects at transcriptional or post-transcriptional levels. Data from other systems have indicated that some elements of the dsRNA response may affect mRNA directly (reviewed in refs **1** and **6**). We therefore sought to develop a cell-free assay that reflected, at least in part, RNAi.

[0159] S2 cells were transfected with dsRNAs corresponding to either cyclin E or lacZ. Cellular extracts were incubated with synthetic mRNAs of lacZ or cyclin E. Extracts prepared from cells transfected with the 540-nucleotide cyclin E dsRNA efficiently degraded the cyclin E transcript; however, the lacZ transcript was stable in these lysates (**FIG. 2***a*). Conversely, lysates from cells transfected with the lacZ dsRNA degraded the lacZ transcript but left the cyclin E mRNA intact. These results indicate that RNAi ablates target mRNAs through the generation of a sequence-specific nuclease activity. We have termed this enzyme RISC (RNA-induced silencing complex). Although we occasionally observed possible intermediates in the degradation process (see **FIG. 2**), the absence of stable cleavage end-products indicates an exonuclease (perhaps coupled to an endonuclease). However, it is possible that the RNAi nuclease makes an initial endonucleolytic cut and that non-specific exonucleases in the extract complete the degradation process[16]. In addition, our ability to create an extract that targets lacZ in vitro indicates that the presence of an endogenous gene is not required for the RNAi response.

[0160] To examine the substrate requirements for the dsRNA-induced, sequence-specific nuclease activity, we incubated a variety of cyclin-E-derived transcripts with an extract derived from cells that had been transfected with the 540-nucleotide cyclin E dsRNA (**FIG. 2***b, c*). Just as a length requirement was observed for the transfected dsRNA, the RNAi nuclease activity showed a dependence on the size of the RNA substrate. Both a 600-nucleotide transcript that extends slightly beyond the targeted region (**FIG. 2***b*) and an ~1-kilobase (kb) transcript that contains the entire coding sequence (data not shown) were completely destroyed by the extract. Surprisingly, shorter substrates were not degraded as

efficiently. Reduced activity was observed against either a 300- or a 220-nucleotide transcript, and a 100-nucleotide transcript was resistant to nuclease in our assay. This was not due solely to position effects because ~100-nucleotide transcripts derived from other portions of the transfected dsRNA behaved similarly (data not shown). As expected, the nuclease activity (or activities) present in the extract could also recognize the antisense strand of the cyclin E mRNA. Again, substrates that contained a substantial portion of the targeted region were degraded efficiently whereas those that contained a shorter stretch of homologous sequence (~130 nucleotides) were recognized inefficiently (**FIG. 2***c*, as600). For both the sense and antisense strands, transcripts that had no homology with the transfected dsRNA (**FIG. 2***b*, Eout; **FIG. 2***c*, as300) were not degraded. Although we cannot exclude the possibility that nuclease specificity could have migrated beyond the targeted region, the resistance of transcripts that do not contain homology to the dsRNA is consistent with data from *C. elegans*. Double-stranded RNAs homologous to an upstream cistron have little or no effect on a linked downstream cistron, despite the fact that unprocessed, polycistronic mRNAs can be readily detected[17, 18]. Furthermore, the nuclease was inactive against a dsRNA identical to that used to provoke the RNAi response in vivo (**FIG. 2***b*). In the in vitro system, neither a 5' cap nor a poly(A) tail was required, as such transcripts were degraded as efficiently as uncapped and non-polyadenylated RNAs.

[0161] Gene silencing provoked by dsRNA is sequence specific. A plausible mechanism for determining specificity would be incorporation of nucleic-acid guide sequences into the complexes that accomplish silencing[19]. In accord with this idea, pre-treatment of extracts with a Ca^{2+}-dependent nuclease (micrococcal nuclease) abolished the ability of these extracts to degrade cognate mRNAs (**FIG. 3**). Activity could not be rescued by addition of non-specific RNAs such as yeast transfer RNA. Although micrococcal nuclease can degrade both DNA and RNA, treatment of the extract with DNAse I had no effect (**FIG. 3**). Sequence-specific nuclease activity, however, did require protein (data not shown). Together, our results support the possibility that the RNAi nuclease is a ribonucleoprotein, requiring both RNA and protein components. Biochemical fractionation (see below) is consistent with these components being associated in extract rather than being assembled on the target mRNA after its addition.

[0162] In plants, the phenomenon of co-suppression has been associated with the existence of small (~25-nucleotide) RNAs that correspond to the gene that is being silenced[19]. To address the possibility that a similar RNA might exist in Drosophila and guide the sequence-specific nuclease in the choice of substrate, we partially purified our activity through several fractionation steps. Crude extracts contained both sequence-specific nuclease activity and abundant, heterogeneous RNAs homologous to the transfected dsRNA (**FIGS. 2 and 4***a*). The RNAi nuclease fractionated with ribosomes in a high-speed centrifugation step. Activity could be extracted by treatment with high salt, and ribosomes could be removed by an additional centrifugation step. Chromatography of soluble nuclease over an anion-exchange column resulted in a discrete peak of activity (**FIG. 4***b*, cyclin E). This retained specificity as it was inactive against a heterologous mRNA (**FIG. 4***b*, lacZ). Active fractions also contained an RNA species of 25 nucleotides that is homologous to the cyclin E target (**FIG. 4***b*, northern). The band observed on northern blots may represent a family of discrete RNAs because it could be detected with probes specific for both the sense and antisense cyclin E sequences and with probes derived from distinct segments of the dsRNA (data not shown). At present, we cannot determine whether the 25-nucleotide RNA is present in the nuclease complex in a double-stranded or single-stranded form.

[0163] RNA interference allows an adaptive defence against both exogenous and endogenous dsRNAs, providing something akin to a dsRNA immune response. Our data, and that of others[19], is consistent with a model in which dsRNAs present in a cell are converted, either through processing or replication, into small specificity determinants of discrete size in a manner analogous to antigen processing. Our results suggest that the post-transcriptional component of dsRNA-dependent gene silencing is accomplished by a sequence-specific nuclease that incorporates these small RNAs as guides that target specific messages based upon sequence recognition. The identical size of putative specificity determinants in plants[19] and animals predicts a conservation of both the mechanisms and the components of dsRNA-induced, post-transcriptional gene silencing in diverse organisms. In plants, dsRNAs provoke not only post-transcriptional gene silencing but also chromatin remodelling and transcriptional repression[20, 21]. It is now critical to determine whether conservation of gene-silencing mechanisms also exists at the transcriptional level and whether chromatin remodelling can be directed in a sequence-specific fashion by these same dsRNA-derived guide sequences.

[0164] Methods

[0165] Cell Culture and RNA Methods

[0166] S2 (ref. 22) cells were cultured at 27° C. in 90% Schneider's insect media (Sigma), 10% heat inactivated fetal bovine serum (FBS). Cells were transfected with dsRNA and plasmid DNA by calcium phosphate co-precipitation[23]. Identical results were observed when cells were transfected using lipid reagents (for example, Superfect, Qiagen). For FACS analysis, cells were additionally transfected with a vector that directs expression of a green fluorescent protein (GFP)-US9 fusion protein[13]. These cells were fixed in 90% ice-cold ethanol and stained with propidium iodide at 25 μg ml^{-1}. FACS was performed on an Elite flow cytometer (Coulter). For northern blotting, equal loading was ensured by over-probing blots with a control complementary DNA (RP49). For the production of dsRNA, transcription templates were generated by polymerase chain reaction such that they contained T7 promoter sequences on each end of the template. RNA was prepared using the RiboMax kit (Promega). Confirmation that RNAs were double stranded came from their complete sensitivity to RNAse III (a gift from A. Nicholson). Target mRNA transcripts were synthesized using the Riboprobe kit (Promega) and were gel purified before use.

[0167] Extract Preparation

[0168] Log-phase S2 cells were plated on 15-cm tissue culture dishes and transfected with 30 μg dsRNA and 30 μg carrier plasmid DNA. Seventy-two hours after transfection, cells were harvested in PBS containing 5 mM EGTA washed twice in PBS and once in hypotonic buffer (10 mM HEPES pH 7.3, 6 mM β-mercaptoethanol). Cells were suspended in

0.7 packed-cell volumes of hypotonic buffer containing Complete protease inhibitors (Boehringer) and 0.5 units ml^{-1} of RNasin (Promega). Cells were disrupted in a dounce homogenizer with a type B pestle, and lysates were centrifuged at 30,000 g for 20 min. Supernatants were used in an in vitro assay containing 20 mM HEPES pH 7.3, 110 mM KOAc, 1 mM $Mg(OAc)_2$, 3 mM EGTA, 2 mM $CaCl_2$, 1 mM DTT. Typically, 5 μl extract was used in a 10 μl assay that contained also 10,000 c.p.m. synthetic mRNA substrate.

[0169] Extract Fractionation

[0170] Extracts were centrifuged at 200,000 g for 3 h and the resulting pellet (containing ribosomes) was extracted in hypotonic buffer containing also 1 mM $MgCl_2$ and 300 mM KOAc. The extracted material was spun at 100,000 g for 1 h and the resulting supernatant was fractionated on Source 15Q column (Pharmacia) using a KCl gradient in buffer A (20 mM HEPES pH 7.0, 1 mM dithiothreitol, 1 mM $MgCl_2$). Fractions were assayed for nuclease activity as described above. For northern blotting, fractions were proteinase K/SDS treated, phenol extracted, and resolved on 15% acrylamide 8M urea gels. RNA was electroblotted onto Hybond N+ and probed with strand-specific riboprobes derived from cyclin E mRNA. Hybridization was carried out in 500 mM $NaPO_4$ pH 7.0, 15% formamide, 7% SDS, 1% BSA. Blots were washed in 1 SSC at 37-45° C.

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Example 2

Role for a Bidentate Ribonuclease in the Initiation Step of RNA Interference

[0194] Genetic approaches in worms, fungi and plants have identified a group of proteins that are essential for double-stranded RNA-induced gene silencing. Among these are ARGONAUTE family members (e.g. RDE1, QDE2)[9, 10,30], recQ-family helicases (MUT-7, QDE3)[11,12], and RNA-dependent RNA polymerases (e.g. EGO-1, QDE1, SGS2/SDE1)[13-16]. While potential roles have been proposed, none of these genes has been assigned a definitive function in the silencing process. Biochemical studies have suggested that PTGS is accomplished by a multicomponent nuclease that targets mRNAs for degradation[6,8,17]. We have shown that the specificity of this complex may derive from the incorporation of a small guide sequence that is homologous to the mRNA substrate[6]. Originally identified in plants that were actively silencing transgenes[7], these ~22 nt. RNAs have been produced during RNAi in vitro using an extract prepared from Drosophila embryos[8]. Putative guide RNAs can also be produced in extracts from Drosophila S2 cells (**FIG. 5***a*). With the goal of understanding the mechanism of post-transcriptional gene silencing, we have undertaken both biochemical fractionation and candidate gene approaches to identify the enzymes that execute each step of RNAi.

[0195] Our previous studies resulted in the partial purification of a nuclease, RISC, that is an effector of RNA interference. See Example 1. This enzyme was isolated from Drosophila S2 cells in which RNAi had been initiated in vivo by transfection with dsRNA. We first sought to determine whether the RISC enzyme and the enzyme that initiates RNAi via processing of dsRNA into 22 mers are distinct activities. RISC activity could be largely cleared from extracts by high-speed centrifugation (100,000×g for 60 min.) while the activity that produces 22 mers remained in the supernatant (**FIG. 5***b,c*). This simple fractionation indicated that RISC and the 22 mer-generating activity are separable and thus distinct enzymes. However, it seems likely that they might interact at some point during the silencing process.

[0196] RNAse III family members are among the few nucleases that show specificity for double-stranded RNA[18]. Analysis of the Drosophila and *C. elegans* genomes reveals several types of RNAse III enzymes. First is the canonical RNAse III which contains a single RNAse III signature motif and a double-stranded RNA binding domain (dsRBD; e.g. RNC_CAEEL). Second is a class represented by Drosha[19], a Drosophila enzyme that contains two RNAse III motifs and a dsRBD (CeDrosha in *C. elegans*). A third class contains two RNAse III signatures and an amino terminal helicase domain (e.g. Drosophila CG4792, CG6493, *C. elegans* K12H4.8), and these had previously been proposed by Bass as candidate RNAi nucleases[20]. Representatives of all three classes were tested for the ability to produce discrete, ~22 nt. RNAs from dsRNA substrates.

[0197] Partial digestion of a 500 nt. cyclin E dsRNA with purified, bacterial RNAse III produced a smear of products while nearly complete digestion produced a heterogeneous group of ~11-17 nucleotide RNAs (not shown). In order to test the dual-RNAse III enzymes, we prepared T7 epitope-tagged versions of Drosha and CG4792. These were expressed in transfected S2 cells and isolated by immunoprecipitation using antibody-agarose conjugates. Treatment of the dsRNA with the CG4792 immunoprecipitate yielded ~22 nt. fragments similar to those produced in either S2 or embryo extracts (**FIG. 6***a*). Neither activity in extract nor activity in immunoprecipitates depended on the sequence of the RNA substrate since dsRNAs derived from several genes were processed equivalently (see Supplement 1). Negative results were obtained with Drosha and with immunoprecipitates of a DExH box helicase (Homeless[21]; see **FIGS. 6***a,b*). Western blotting confirmed that each of the tagged proteins was expressed and immunoprecipitated similarly (see Supplement 2). Thus, we conclude that CG4792 may carry out the initiation step of RNA interference by producing ~22 nt. guide sequences from dsRNAs. Because of its ability to digest dsRNA into uniformly sized, small RNAs, we have named this enzyme Dicer (Dcr). Dicer mRNA is expressed in embryos, in S2 cells, and in adult flies, consistent with the presence of functional RNAi machinery in all of these contexts (see Supplement 3).

[0198] The possibility that Dicer might be the nuclease responsible for the production of guide RNAs from dsRNAs prompted us to raise an antiserum directed against the carboxy-terminus of the Dicer protein (Dicer-1, CG4792). This antiserum could immunoprecipitate a nuclease activity from either Drosophila embryo extracts or from S2 cell lysates that produced ~22 nt. RNAs from dsRNA substrates (**FIG. 6C**). The putative guide RNAs that are produced by the Dicer-1 enzyme precisely comigrate with 22 mers that are produced in extract and with 22 mers that are associated with the RISC enzyme (**FIGS. 6 D,F**). It had previously been shown that the enzyme that produced guide RNAs in Drosophila embryo extracts was ATP-dependent[6]. Depletion of this cofactor resulted in an ~6-fold lower rate of dsRNA cleavage and in the production of RNAs with a slightly lower mobility. Of interest was the fact that both Dicer-1 immunoprecipitates and extracts from S2 cells require ATP for the production of 22 mers (**FIG. 6D**). We do not observe the accumulation of lower mobility products in these cases, although we do routinely observe these in ATP-depleted embryo extracts. The requirement of this nuclease for ATP is a quite unusual property. We hypothesize that this requirement could indicate that the enzyme may act processively on the dsRNA, with the helicase domain harnessing the energy of ATP hydrolysis both for unwinding guide RNAs and for translocation along the substrate.

[0199] Efficient induction of RNA interference in *C. elegans* and in Drosophila has several requirements. For example, the initiating RNA must be double-stranded, and it must be several hundred nucleotides in length. To determine whether these requirements are dictated by Dicer, we characterized the ability of extracts and of immunoprecipitated enzyme to digest various RNA substrates. Dicer was inactive against single stranded RNAs regardless of length (see Supplement 4). The enzyme could digest both 200 and 500 nucleotide dsRNAs but was significantly less active with shorter substrates (see Supplement 4). Double-stranded RNAs as short as 35 nucleotides could be cut by the enzyme, albeit very inefficiently (data not shown). In contrast, *E. coli* RNAse III could digest to completion dsRNAs of 35 or 22 nucleotides (not shown). This suggests that the substrate preferences of the Dicer enzyme may contribute to but not wholly determine the size dependence of RNAi.

[0200] To determine whether the Dicer enzyme indeed played a role in RNAi in vivo, we sought to deplete Dicer activity from S2 cells and test the effect on dsRNA-induced gene silencing. Transfection of S2 cells with a mixture of dsRNAs homologous to the two Drosophila Dicer genes (CG4792 and CG6493) resulted in an ~6-7 fold reduction of Dicer activity either in whole cell lysates or in Dicer-1 immunoprecipitates (**FIGS. 7A,B**). Transfection with a control dsRNA (murine caspase 9) had no effect. Qualitatively similar results were seen if Dicer was examined by Northern blotting (not shown). Depletion of Dicer in this manner substantially compromised the ability of cells to silence subsequently an exogenous, GFP transgene by RNAi (**FIG. 7C**). These results indicate that Dicer is involved in RNAi in vivo. The lack of complete inhibition of silencing could result from an incomplete suppression of Dicer (which is itself required for RNAi) or could indicate that in vivo, guide RNAs can be produced by more than one mechanism (e.g. through the action of RNA-dependent RNA polymerases).

[0201] Our results indicate that the process of RNA interference can be divided into at least two distinct steps. According to this model, initiation of PTGS would occur upon processing of a double-stranded RNA by Dicer into ~22 nucleotide guide sequences, although we cannot formally exclude the possibility that another, Dicer-associated nuclease may participate in this process. These guide RNAs would be incorporated into a distinct nuclease complex (RISC) that targets single-stranded mRNAs for degradation. An implication of this model is that guide sequences are themselves derived directly from the dsRNA that triggers the response. In accord with this model, we have demonstrated that ^{32}P-labeled, exogenous dsRNAs that have been introduced into S2 cells by transfection are incorporated into the RISC enzyme as 22 mers (**FIG. 7E**). However, we cannot exclude the possibility that RNA-dependent RNA polymerases might amplify 22 mers once they have been generated or provide an alternative method for producing guide RNAs.

[0202] The structure of the Dicer enzyme provokes speculation on the mechanism by which the enzyme might produce discretely sized fragments irrespective of the sequence of the dsRNA (see Supplement 1, **FIG. 8*a***). It has been established that bacterial RNAse III acts on its substrate as a dimer[18,22,23]. Similarly, a dimer of Dicer enzymes may be required for cleavage of dsRNAs into ~22 nt. pieces. According to one model, the cleavage interval would be determined by the physical arrangement of the two RNAse III domains within Dicer enzyme (**FIG. 8*a***). A plausible alternative model would dictate that cleavage was directed at a single position by the two RIII domains in a single Dicer protein. The 22 nucleotide interval could be dictated by interaction of neighboring Dicer enzymes or by translocation along the mRNA substrate. The presence of an integral helicase domain suggests that the products of Dicer cleavage might be single-stranded 22 mers that are incorporated into the RISC enzyme as such.

[0203] A notable feature of the Dicer family is its evolutionary conservation. Homologs are found in *C. elegans* (K12H4.8), Arabidopsis (e.g., CARPEL FACTORY[24], T25K16.4, AC012328_1), mammals (Helicase-MOI[25]) and *S. pombe* (YC9A_SCHPO) (**FIG. 8*b***, see Supplements 6,7 for sequence comparisons). In fact, the human Dicer family member is capable of generating ~22 nt. RNAs from dsRNA substrates (Supplement 5) suggesting that these structurally similar proteins may all share similar biochemical functions. It has been demonstrated that exogenous dsRNAs can affect gene function in early mouse embryos[29], and our results suggest that this regulation may be accomplished by an evolutionarily conserved RNAi machinery.

[0204] In addition to RNAseIII and helicase motifs, searches of the PFAM database indicate that each Dicer family member also contains a ZAP domain (**FIG. 8*c***)[27]. This sequence was defined based solely upon its conservation in the Zwille/ARGONAUTE/Piwi family that has been implicated in RNAi by mutations in *C. elegans* (Rde-1)[9] and Neurospora (Qde-2)[10]. Although the function of this domain is unknown, it is intriguing that this region of homology is restricted to two gene families that participate in dsRNA-dependent silencing. Both the ARGONAUTE and Dicer families have also been implicated in common biological processes, namely the determination of stem-cell fates. A hypomorphic allele of carpel factory, a member of the Dicer family in Arabidopsis, is characterized by increased proliferation in floral meristems[24]. This phenotype and a number of other characteristic features are also shared by Arabidopsis ARGONAUTE (ago1-1) mutants[26] (C. Kidner and R. Martienssen, pers. comm.). These genetic analyses begin to provide evidence that RNAi may be more than a defensive response to unusual RNAs but may also play important roles in the regulation of endogenous genes.

[0205] With the identification of Dicer as a catalyst of the initiation step of RNAi, we have begun to unravel the biochemical basis of this unusual mechanism of gene regulation. It will be of critical importance to determine whether the conserved family members from other organisms, particularly mammals, also play a role in dsRNA-mediated gene regulation.

[0206] Methods

[0207] Plasmid constructs. A full-length cDNA encoding Drosha was obtained by PCR from an EST sequenced by the Berkeley Drosophila genome project. The Homeless clone was a gift from Gillespie and Berg (Univ. Washington). The T7 epitope-tag was added to the amino terminus of each by PCR, and the tagged cDNAs were cloned into pRIP, a retroviral vector designed specifically for expression in insect cells (E. Bernstein, unpublished). In this vector, expression is driven by the *Orgyia pseudotsugata* IE2 promoter (Invitrogen). Since no cDNA was available for CG4792/Dicer, a genomic clone was amplified from a bacmid (BACR23F10; obtained from the BACPAC Resource Center in the Dept. of Human Genetics at the Roswell Park Cancer Institute). Again, during amplification, a T7 epitope tag was added at the amino terminus of the coding sequence. The human Dicer gene was isolated from a cDNA library prepared from HaCaT cells (GJH, unpublished). A T7-tagged version of the complete coding sequence was cloned into pCDNA3 (Invitrogen) for expression in human cells (LinX-A).

[0208] Cell culture and extract preparation. S2 and embryo culture. S2 cells were cultured at 27° C. in 5% CO_2 in Schneider's insect media supplemented with 10% heat inactivated fetal bovine serum (Gemini) and 1% antibiotic-antimycotic solution (Gibco BRL). Cells were harvested for extract preparation at 10×10^6 cells/ml. The cells were washed 1× in PBS and were resuspended in a hypotonic

buffer (10 mM Hepes pH 7.0, 2 mM MgCl2, 6 mM βME) and dounced. Cell lysates were spun 20,000xg for 20 minutes. Extracts were stored at −80° C. Drosophila embryos were reared in fly cages by standard methodologies and were collected every 12 hours. The embryos were dechorionated in 50% chlorox bleach and washed thoroughly with distilled water. Lysis buffer (10 mM Hepes, 10 mM KCl, 1.5 mM $MgCl_2$, 0.5 mM EGTA, 10 mM β-glycerophosphate, 1 mM DTT, 0.2 mM PMSF) was added to the embryos, and extracts were prepared by homogenization in a tissue grinder. Lysates were spun for two hours at 200,000xg and were frozen at −80° C. LinX-A cells, a highly-transfectable derivative of human 293 cells, (Lin Xie and GJH, unpublished) were maintained in DMEM/10%FCS.

[0209] Transfections and immunoprecipitations. S2 cells were transfected using a calcium phosphate procedure essentially as previously described[6]. Transfection rates were ~90% as monitored in controls using an in situ β-galactosidase assay. LinX-A cells were also transfected by calcium phosphate co-precipitation. For immunoprecipitations, cells (~$5x10^6$ per IP) were transfected with various clones and lysed three days later in IP buffer (125 mM KOAc, 1 mM MgOAc, 1 mM $CaCl_2$, 5 mM EGTA, 20 mM Hepes pH 7.0, 1 mM DTT, 1% NP-40 plus Complete protease inhibitors (Roche)). Lysates were spun for 10 minutes at 14,000×g and supernatants were added to T7 antibody-agarose beads (Novagen). Antibody binding proceeded for 4 hours at 4° C. Beads were centrifuged and washed in lysis buffer three times, and once in reaction buffer. The Dicer antiserum was raised in rabbits using a KLH-conjugated peptide corresponding to the C-terminal 8 amino acids of Drosophila Dicer-1 (CG4792).

[0210] Cleavage reactions. RNA preparation. Templates to be transcribed into dsRNA were generated by PCR with forward and reverse primers, each containing a T7 promoter sequence. RNAs were produced using Riboprobe (Promega) kits and were uniformly labeling during the transcription reaction with ^{32}P-UTP. Single-stranded RNAs were purified from 1% agarose gels. dsRNA cleavage. Five microliters of embryo or S2 extracts were incubated for one hour at 30° C. with dsRNA in a reaction containing 20 mM Hepes pH 7.0, 2 mM MgOAc, 2 mM DTT, 1 mM ATP and 5% Superasin (Ambion). Immunoprecipitates were treated similarly except that a minimal volume of reaction buffer (including ATP and Superasin) and dsRNA were added to beads that had been washed in reaction buffer (see above). For ATP depletion, Drosophila embryo extracts were incubated for 20 minutes at 30° C. with 2 mM glucose and 0.375 U of hexokinase (Roche) prior to the addition of dsRNA.

[0211] Northern and Western analysis. Total RNA was prepared from Drosophila embryos (0-12 hour), from adult flies, and from S2 cells using Trizol (Lifetech). Messenger RNA was isolated by affinity selection using magnetic oligo-dT beads (Dynal). RNAs were electrophoresed on denaturing formaldehyde/agarose gels, blotted and probed with randomly primed DNAs corresponding to Dicer. For Western analysis, T7-tagged proteins were immunoprecipitated from whole cell lysates in IP buffer using anti-T7-antibody-agarose conjugates. Proteins were released from the beads by boiling in Laemmli buffer and were separated by electrophoresis on 8% SDS PAGE. Following transfer to nitrocellulose, proteins were visualized using an HRP-conjugated anti-T7 antibody (Novagen) and chemiluminescent detection (Supersignal, Pierce).

[0212] RNAi of Dicer. Drosophila S2 cells were transfected either with a dsRNA corresponding to mouse caspase 9 or with a mixture of two dsRNAs corresponding to Drosophila Dicer-1 and Dicer-2 (CG4792 and CG6493). Two days after the initial transfection, cells were again transfected with a mixture containing a GFP expression plasmid and either luciferase dsRNA or GFP dsRNA as previously described[6]. Cells were assayed for Dicer activity or fluorescence three days after the second transfection. Quantification of fluorescent cells was done on a Coulter EPICS cell sorter after fixation. Control transfections indicated that Dicer activity was not affected by the introduction of caspase 9 dsRNA.

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29. In this regard, the now pending claims of the Hannon '086 application are directed to one particularly valuable aspect of the technology Dr. Hannon developed, methods that allow one to stably suppress gene expression in mammalian cells using RNA interference. Among other things, this valuable technology provides an efficient, effective and widely applicable alternative to more expensive and laborious methods for biomedical research and drug development. Dr. Hannon's shRNA methods represented a considerable advance over the prior art, including the Fire patent, which failed to provide any solution for how to use RNA interference in mammals, without killing the treated cells through the so-called "interferon" or "protein kinase (PK) response".

30. Vincent first added claims directed to use of hairpin RNAs to inhibit gene expression and expression of such hairpin RNAs in cells of a transgenic non-human mammal in the '557 application, which was filed May 24, 2001 as a continuation-in-part ("CIP") of the '435 PCT application. Both from the standpoint of meeting his duty of care and scientifically, in adding these new claims, Vincent had an affirmative duty to amend the specification with original text accurately describing these additional claimed inventions and distinguish these from the Fire application. However, instead of properly amending the Detailed Description, Vincent again relied on the same text he had previously copied from the Fire application as support for these new claims, knowing full well that text copied directly from Fire could not serve to distinguish the newly claimed subject matter from Fire.

31. During 2001, Vincent regularly communicated with Dr. Hannon regarding the Hannon Applications Vincent was then prosecuting. In filing the '557 application on May 24, 2001, and also in filing the '797 application in January 22, 2002, Vincent either knew or should have known the relevance and potential prejudice of continuing to rely on extensive

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[0239] 27. Sonnhammer, E. L., Eddy, S. R. & Durbin, R. Pfam: a comprehensive database of protein domain families based on seed alignments. *Proteins* 28, 405-20 (1997).

[0240] 28. Altschul, S. F. et al. Gapped BLAST and PSI-BLAST: a new generation of protein database search programs. *Nucleic Acids Res* 25, 3389-402 (1997).

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Example 3

A simplified Method for the Creation of Hairpin Constructs for RNA Interference

[0243] In numerous model organisms, double stranded RNAs have been shown to cause effective and specific suppression of gene function (ref. 1). This response, termed RNA interference or post-transcriptional gene silencing, has evolved into a highly effective reverse genetic tool in *C. elegans*, Drosophila, plants and numerous other systems. In these cases, double-stranded RNAs can be introduced by injection, transfection or feeding; however, in all cases, the response is both transient and systemic. Recently, stable interference with gene expression has been achieved by expression of RNAs that form snap-back or hairpin structures (refs 2-7). This has the potential not only to allow stable silencing of gene expression but also inducible silencing as has been observed in trypanosomes and adult Drosophila (refs 2,4,5). The utility of this approach is somewhat hampered by the difficulties that arise in the construction of bacterial plasmids containing the long inverted repeats that are necessary to provoke silencing. In a recent report, it was stated that more than 1,000 putative clones were screed to identify the desired construct (ref 7).

[0244] The presence of hairpin structures often induces plasmid rearrangement, in part due to the *E. coli* sbc proteins that recognize and cleave cruciform DNA structures (ref 8). We have developed a method for the construction of hairpins that does not require cloning of inverted repeats, per se. Instead, the fragment of the gene that is to be silenced is cloned as a direct repeat, and the inversion is accomplished by treatment with a site-specific recombinase, either in vitro (or potentially in vivo) (see **FIG. 27**). Following recombination, the inverted repeat structure is stable in a bacterial strain that lacks an intact SBC system (DL759). We have successfully used this strategy to construct numerous hairpin expression constructs that have been successfully used to provoke gene silencing in Drosophila cells.

Literature Cited in Example 3

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V. EQUIVALENTS

[0253] Those skilled in the art will recognize, or be able to ascertain using no more than routine experimentation, many equivalents to the specific embodiments of the invention described herein. Such equivalents are intended to be encompassed by the following claims.

[0254] All of the above-cited references and publications are hereby incorporated by reference.

We claim:

1. A method for attenuating expression of a target gene in cultured cells, comprising introducing double stranded RNA (dsRNA) into the cells in an amount sufficient to attenuate expression of the target gene, wherein the dsRNA comprises a nucleotide sequence that hybridizes under stringent conditions to a nucleotide sequence of the target gene.

2. A method for attenuating expression of a target gene in a mammalian cell, comprising

(i) activating one or both of a Dicer activity or an Argonaut activity in the cell, and

(ii) introducing into the cell a double stranded RNA (dsRNA) in an amount sufficient to attenuate expression of the target gene, wherein the dsRNA comprises a nucleotide sequence that hybridizes under stringent conditions to a nucleotide sequence of the target gene.

3. The method of claim 2, wherein the cell is suspended in culture.

4. The method of claim 2, wherein the cell is in a whole animal, such as a non-human mammal.

5. The method of claim 1 or 2, wherein is engineered with (i) a recombinant gene encoding a Dicer activity, (ii) a recombinant gene encoding an Argonaut activity, or (iii) both.

6. The method of claim 5, wherein the recombinant gene encodes a protein which includes an amino acid sequence at least 50 percent identical to SEQ ID No. 2 or 4 or the Argonaut sequence shown in FIG. 24.

7. The method of claim 5, wherein the recombinant gene includes a coding sequence hybridizes under wash conditions of 2×SSC at 22° C. to SEQ ID No. 1 or 3.

8. The method of claim 1 or 2, wherein an endogenous Dicer gene or Argonaut gene is activated.

9. The method of claim 1 or 2, wherein the target gene is an endogenous gene of the cell.

10. The method of claim 1 or 2, wherein the target gene is an heterologous gene relative to the genome of the cell, such as a pathogen gene.

11. The method of claim 1 or 2, wherein the cell is treated with an agent that inhibits protein kinase RNA-activated (PKR) apoptosis, such as by treatment with agents which inhibit expression of PKR, cause its destruction, and/or inhibit the kinase activity of PKF.

12. The method of claim 1 or 2, wherein the cell is a primate cell, such as a human cell.

13. The method of claim 1 or 2, wherein the dsRNA is at least 20 nucleotides in length.

14. The method of claim 13, wherein the dsRNA is at least 100 nucleotides in length.

15. The method of claim 1 or 2, wherein expression of the target gene is attenuated by at least 10 fold.

16. An assay for identifying nucleic acid sequences responsible for conferring a particular phenotype in a cell, comprising

(i) constructing a variegated library of nucleic acid sequences from a cell in an orientation relative to a promoter to produce double stranded DNA;

(ii) introducing the variegated dsRNA library into a culture of target cells, which cells have an activated Dicer activity or Argonaut activity;

(iii) identifying members of the library which confer a particular phenotype on the cell, and identifying the sequence from a cell which correspond, such as being identical or homologous, to the library member.

17. A method of conducting a drug discovery business comprising:

(i) identifying, by the assay of claim 16, a target gene which provides a phenotypically desirable response when inhibited by RNAi;

(ii) identifying agents by their ability to inhibit expression of the target gene or the activity of an expression product of the target gene;

(iii) conducting therapeutic profiling of agents identified in step (b), or further analogs thereof, for efficacy and toxicity in animals; and

(iv) formulating a pharmaceutical preparation including one or more agents identified in step (iii) as having an acceptable therapeutic profile.

18. The method of claim 17, including an additional step of establishing a distribution system for distributing the pharmaceutical preparation for sale, and may optionally include establishing a sales group for marketing the pharmaceutical preparation.

19. A method of conducting a target discovery business comprising:

(i) identifying, by the assay of claim 16, a target gene which provides a phenotypically desirable response when inhibited by RNAi;

JS 44 (Rev. 12/07)

CIVIL COVER SHEET

The JS 44 civil cover sheet and the information contained herein neither replace nor supplement the filing and service of pleadings or other papers as required by law, except as provided by local rules of court. This form, approved by the Judicial Conference of the United States in September 1974, is required for the use of the Clerk of Court for the purpose of initiating the civil docket sheet. (SEE INSTRUCTIONS ON THE REVERSE OF THE FORM.)

I. (a) PLAINTIFFS
COLD SPRING HARBOR LABORATORY

(b) County of Residence of First Listed Plaintiff Cold Spring Harbor, NY
(EXCEPT IN U.S. PLAINTIFF CASES)

(c) Attorney's (Firm Name, Address, and Telephone Number)
SCULLY, SCOTT, MURPHY & PRESSER P.C.

DEFENDANTS
ROPES & GRAY LLP and MATTHEW P. VINCENT

County of Residence of First Listed Defendant Boston, Massachusetts
(IN U.S. PLAINTIFF CASES ONLY)
NOTE: IN LAND CONDEMNATION CASES, USE THE LOCATION OF THE LAND INVOLVED.

Attorneys (If Known) Philip R. Forlenza, Esq.
Patterson Belknap Webb & Tyler LLP

II. BASIS OF JURISDICTION (Place an "X" in One Box Only)

- ☐ 1 U.S. Government Plaintiff
- ☒ 3 Federal Question (U.S. Government Not a Party)
- ☐ 2 U.S. Government Defendant
- ☐ 4 Diversity (Indicate Citizenship of Parties in Item III)

III. CITIZENSHIP OF PRINCIPAL PARTIES (Place an "X" in One Box for Plaintiff and One Box for Defendant)
(For Diversity Cases Only)

	PTF	DEF		PTF	DEF
Citizen of This State	☒ 1	☐ 1	Incorporated or Principal Place of Business In This State	☐ 4	☐ 4
Citizen of Another State	☐ 2	☐ 2	Incorporated and Principal Place of Business In Another State	☐ 5	☒ 5
Citizen or Subject of a Foreign Country	☐ 3	☐ 3	Foreign Nation	☐ 6	☐ 6

CV - 10 0661

IV. NATURE OF SUIT (Place an "X" in One Box Only)

☐ 110 Insurance ☐ 120 Marine ☐ 130 Miller Act ☐ 140 Negotiable Instrument ☐ 150 Recovery of Overpayment & Enforcement of Judgment ☐ 151 Medicare Act ☐ 152 Recovery of Defaulted Student Loans (Excl. Veterans) ☐ 153 Recovery of Overpayment of Veteran's Benefits ☐ 160 Stockholders' Suits ☐ 190 Other Contract ☐ 195 Contract Product Liability ☐ 196 Franchise	**PERSONAL INJURY** ☐ 310 Airplane ☐ 315 Airplane Product Liability ☐ 320 Assault, Libel & Slander ☐ 330 Federal Employers' Liability ☐ 340 Marine ☐ 345 Marine Product Liability ☐ 350 Motor Vehicle ☐ 355 Motor Vehicle Product Liability ☐ 360 Other Personal Injury	**PERSONAL INJURY** ☐ 362 Personal Injury - Med. Malpractice ☐ 365 Personal Injury - Product Liability ☐ 368 Asbestos Personal Injury Product Liability **PERSONAL PROPERTY** ☐ 370 Other Fraud ☐ 371 Truth in Lending ☐ 380 Other Personal Property Damage ☐ 385 Property Damage Product Liability	☐ 610 Agriculture ☐ 620 Other Food & Drug ☐ 625 Drug Related Seizure of Property 21 USC 881 ☐ 630 Liquor Laws ☐ 640 R.R. & Truck ☐ 650 Airline Regs. ☐ 660 Occupational Safety/Health ☐ 690 Other	☐ 422 Appeal 28 USC 158 ☐ 423 Withdrawal 28 USC 157 ☐ 820 Copyrights ☐ 830 Patent ☐ 840 Trademark	☐ 400 State Reapportionment ☐ 410 Antitrust ☐ 430 Banks and Banking ☐ 450 Commerce ☐ 460 Deportation ☐ 470 Racketeer Influenced and Corrupt Organizations ☐ 480 Consumer Credit ☐ 490 Cable/Sat TV ☐ 810 Selective Service ☐ 850 Securities/Commodities/ Exchange ☐ 875 Customer Challenge 12 USC 3410 ☐ 890 Other Statutory Actions ☐ 891 Agricultural Acts ☐ 892 Economic Stabilization Act ☐ 893 Environmental Matters ☐ 894 Energy Allocation Act ☐ 895 Freedom of Information Act ☐ 900Appeal of Fee Determination Under Equal Access to Justice ☐ 950 Constitutionality of State Statutes
☐ 210 Land Condemnation ☐ 220 Foreclosure ☐ 230 Rent Lease & Ejectment ☐ 240 Torts to Land ☐ 245 Tort Product Liability ☐ 290 All Other Real Property	☐ 441 Voting ☐ 442 Employment ☐ 443 Housing/ Accommodations ☐ 444 Welfare ☐ 445 Amer. w/Disabilities - Employment ☐ 446 Amer. w/Disabilities - Other ☐ 440 Other Civil Rights	☐ 510 Motions to Vacate Sentence **Habeas Corpus:** ☐ 530 General ☐ 535 Death Penalty ☐ 540 Mandamus & Other ☐ 550 Civil Rights ☐ 555 Prison Condition	☐ 462 Naturalization Application ☐ 463 Habeas Corpus - Alien Detainee ☐ 465 Other Immigration Actions	☐ 861 HIA (1395ff) ☐ 862 Black Lung (923) ☐ 863 DIWC/DIWW (405(g)) ☐ 864 SSID Title XVI ☐ 865 RSI (405(g)) ☐ 870 Taxes (U.S. Plaintiff or Defendant) ☐ 871 IRS—Third Party 26 USC 7609	

SPATT J.
TOMLINSON M.J.

V. ORIGIN (Place an "X" in One Box Only)
☒ 1 Original Proceeding ☐ 2 Removed from State Court ☐ 3 Remanded from Appellate Court ☐ 4 Reinstated or Reopened ☐ 5 Transferred from another district (specify) ☐ 6 Multidistrict Litigation ☐ 7 Appeal to District Judge from Magistrate Judgment

VI. CAUSE OF ACTION
Cite the U.S. Civil Statute under which you are filing (Do not cite jurisdictional statutes unless diversity):
Brief description of cause:
negligence / malpractice in prosecution of patent applications

VII. REQUESTED IN COMPLAINT:
☐ CHECK IF THIS IS A CLASS ACTION UNDER F.R.C.P. 23
DEMAND $
CHECK YES only if demanded in complaint:
JURY DEMAND: ☒ Yes ☐ No

VIII. RELATED CASE(S) IF ANY (See instructions): JUDGE _____ DOCKET NUMBER _____

DATE
02/16/2010

SIGNATURE OF ATTORNEY OF RECORD

FOR OFFICE USE ONLY

RECEIPT # _____ AMOUNT _____ APPLYING IFP _____ JUDGE _____ MAG. JUDGE _____

ARBITRATION CERTIFICATION

I, Peter I. Bernstein, counsel for Cold Spring Harbor Laboratory do hereby certify pursuant to the Local Arbitration Rule 83.10 that to the best of my knowledge and belief the damages recoverable in the above captioned civil action exceed the sum of $150,000 exclusive of interest and costs. _____ Relief other than monetary damages is sought.

DISCLOSURE STATEMENT - FEDERAL RULES CIVIL PROCEDURE 7.1

Identify any parent corporation and any publicly held corporation that owns 10% or more or its stocks:

RELATED CASE STATEMENT (SECTION VIII)

All cases that are arguably related pursuant to Division of Business Rule 50.3.1 should be listed in Section VIII on the front of this form. Rule 50.3.1 (a) provides that "A civil case is "related" to another civil case for purposes of this guideline when, because of the similarity of facts and legal issues or because the cases arise from the same transactions or events, a substantial saving of judicial resources is likely to result from assigning both cases to the same judge and magistrate judge."

NY-E DIVISION OF BUSINESS RULE 50.1(d)(2)

1.) Is the civil action being filed in the Eastern District removed from a New York State Court located in Nassau or Suffolk County: No

2.) If you answered "no" above:

a) Did the events or omissions giving rise to the claim or claims, or a substantial part thereof, occur in Nassau or Suffolk County? Yes

b) Did the events of omissions giving rise to the claim or claims, or a substantial part thereof, occur in the Eastern District? Yes

If your answer to question 2 (b) is "No," does the defendant (or a majority of the defendants, if there is more than one) reside in Nassau or Suffolk County, or, in an interpleader action, does the claimant (or a majority of the claimants, if there is more than one) reside in Nassau or Suffolk County? _____

(Note: A corporation shall be considered a resident of the County in which it has the most significant contacts).

BAR ADMISSION

I am currently admitted in the Eastern District of New York and currently a member in good standing of the bar of this court.

Yes X No _____

Are you currently the subject of any disciplinary action (s) in this or any other state or federal court?

Yes _____ (If yes, please explain) No X

_____

_____

_____

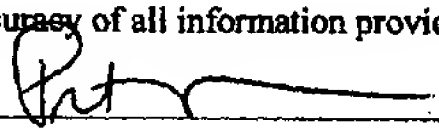
Please provide your E-MAIL address and bar code below. Your bar code consists of the initials of your first and last name and the last four digits of your social security number or any other four digit number registered by the attorney with the Clerk of Court. (This information must be provided pursuant to local rule 11.1(b) of the civil rules).

Attorney Bar Code: 2648681

E-MAIL Address: pibernstein@ssmp.com

Electronic filing procedures were adopted by the Court in Administrative Order No. 97-12, "In re: Electronic Filing Procedures (ECF)." Electronic filing became mandatory in Administrative Order 2004-08, "In re: Electronic Case Filing." Electronic service of all papers is now routine.

I certify the accuracy of all information provided above.

Signature: 

passages of text copied from the Fire application. He should have told Dr. Hannon then what he had done. However, before filing the '557 application, and even the '797 application, Vincent failed to inform Dr. Hannon and CSHL (either directly or through Dr. Hannon) of this copying of Fire and Vincent's continued use of this copied text in the Detailed Description as support for the filed claims. His failure to do so deprived Dr. Hannon and CSHL of the timely opportunity to amend the specification to properly describe and distinguish Dr. Hannon's technology from the different methods Fire described.

32. As corroborated by laboratory and other records, by the time Vincent had filed the '557 application, Dr. Hannon had already conceived of the short hairpin methods that are the subject of the now pending Hannon claims. Had Vincent in May 2001 informed CSHL and Dr. Hannon of his conduct, such information would have identified an urgent need to amend the '557 application to distinguish Dr. Hannon's hairpin technology, including in particular the use of short hairpins, from Fire's altogether different disclosure. Such amendment would necessarily have included adding original disclosure describing Dr. Hannon's short hairpin invention.

33. Vincent's failure to inform CSHL and Dr. Hannon of his conduct resulted in an entirely unnecessary and prejudicial delay in adding specific disclosure about the short hairpin invention to the Hannon Applications. To the extent Vincent eventually did so, this happened eight months later with the filing of the '797 CIP application on January 22, 2002. Even then, instead of revising the Detailed Description to provide an accurate description of the short hairpin technology, Vincent continued to improperly rely on the text he had copied from the Fire application, fully knowing that this text was directed to an entirely different invention. Had Vincent been forthright in May of 2001 about his copying of the Fire text, Vincent would

have no doubt been apprised then (if he was not already aware) of Dr. Hannon's work relating to the short hairpin invention. Instead, by waiting until January 2002 to get reference to short hairpins into Dr. Hannon's applications, Vincent caused a potential crucial loss of priority from May 2001.

34. In short, even after the filing of the '557 application, Vincent and R&G failed to comply with a reasonable standard of care in the subsequent prosecution of these applications and the filing of subsequent applications in the PTO based on these parent applications. Vincent and R&G never brought the fact of Vincent's copying of the Fire text, and the potential prejudice resulting from that copying, to the attention of CSHL. Despite being aware of how his conduct had compromised the Hannon Applications, Vincent continued to prosecute them while hiding this fact from CSHL. Significantly, this deprived CSHL of any opportunity to address the issues the copying raised early in prosecution, when the applications could have been re-drafted in a timely fashion to minimize any potential loss of priority and minimize the harm to CSHL.

35. Further compounding the harm Vincent had caused, in prosecuting these early applications, Vincent's and R&G's improperly relied on and misrepresented the copied Fire text as describing the technology Dr. Hannon invented. In effect, their actions erroneously implied that Dr. Hannon's technology was previously invented or described by Fire, which it was not. Through their failure to properly attribute the copied text to Fire, and years of delay in even bringing the Fire application and Fire Patent to the attention of the PTO, Vincent and R&G further compounded their malpractice by effectively continuing to misrepresent that disclosure as being part of Dr. Hannon's work.

36. For example, these misrepresentations include statements made during prosecution of the '557 application. In the office action dated April 21, 2005 rejecting all pending claims, the PTO Examiner argued that the specification failed to teach introducing an expression vector encoding a hairpin RNA into mammalian cells. In response (Reply and Amendment filed August 11, 2005), R&G argued that the application described the use of expression systems that are intended to produce hairpin RNAs upon being transcribed in cells. In support, R&G repeatedly pointed to various sections of text copied from the Fire application. To support its position, R&G filed a Rule 132 Expert Declaration (Declaration under 35 U.S.C. §1.132 of Frank McKeon dated July 29, 2005), which cited repeatedly to sections of the copied Fire text as evidence that the technology invented by Dr. Hannon and described in the '557 application was directed to use of expression systems intended to produce hairpin RNAs upon being transcribed in cells.

37. As stated above, instead of properly re-drafting the Hannon Applications via the numerous CIP applications, in a way that relied upon an original description of Dr. Hannon's work, Vincent and R&G continued to rely on text copied from Fire despite the fact that this risked the false implication that Dr. Hannon's shRNA technology was either something that Fire invented or was suggested by the Fire application.

38. Notably, on August 11, 2005, the PTO issued a Notice of Allowance for the pending '557 application claims. In explaining his reasons for allowance, the Examiner stated on page 3:

> The declarations under 37 CFR 1.132 filed August 11, 2005 are sufficient to overcome the rejections of claims...based upon new matter under 35 USC 112 first paragraph and lack of enablement under 35 USC 112 first paragraph. Specifically, the declaration of Frank Mckeon establishes that the double stranded RNA construct

> of the patent application encompass hairpin RNA comprising the features claimed therein. The declaration provides evidence by specific examples in the instant application.

39. On April 6, 2006, however, the PTO withdrew the '557 application from issue to reconsider its decision. On September 6, 2006, the Examiner rejected all pending claims as anticipated by the Fire Patent.

40. The prejudice to the Hannon Applications caused by Vincent's original attempt to describe the Hannon inventions by copying text from Fire application was illustrated by R&G's subsequent failed efforts to overcome the Fire Patent as prior art against the '557 application. In the Amendment filed March 9, 2007, Vincent argued that in contrast to the disclosure of the '557 application, the Fire Patent failed to provide any particular guidance that would have led one to envisage the claimed methods directed to using an expression vector encoding a hairpin RNA to attenuate gene expression specifically in mammalian cells.

41. In fact, before filing the March 9, 2007 Amendment, Vincent conducted an in-person interview in February with Examiner McGarry and Supervisory Examiner Schultz at the PTO, specifically to discuss the Examiner McGarry's rejection of the pending claims as anticipated by the Fire Patent. Notably, Vincent brought with him to the interview both Dr. Hannon and John Maroney, the Vice President, Legal Counsel, and Director of CSHL's Office of Technology Transfer. During this interview, Vincent provided the Examiners with a preview of the argument he planned to present in the March 9, 2007 Amendment. Despite the fact that his planned arguments relied on text copied from the Fire specification, Vincent never disclosed this fact to Examiners McGarry and Schultz, Dr. Hannon or Mr. Maroney.

42. In the Office Action dated September 4, 2007, the PTO rejected Vincent's argument that only the '557 specification provided such guidance. Referring specifically to the text that Vincent had copied from Fire, the PTO noted that "in fact the disclosure of cell/organisms of the instant specification at pages 21-22 is essentially verbatim of the disclosure of Fire et al at column 8," and that "it is unclear how applicant claimed invention differs from what has been disclosed by the prior art."

43. The '797 application was filed as a continuation-in-part of the '435 PCT application and among other things, incorporated additional disclosures from Dr. Hannon relating to the use of the shRNA technology for regulating gene expression in mammalian cells. This added material was directed to an entirely different invention from the technology described in the Fire application. Despite that fact, Vincent retained the copied Fire text in the Detailed Description, fully knowing that the copied Fire text described a different invention.

44. In copying Fire again in filing the '797 application, Vincent furthered his malpractice by again failing to make any reasonable effort to amend the specification, in either the summary or detailed description of the invention, to accurately support claims directed to use of short hairpins in mammalian cells. Instead, Vincent continued to rely on the extensively copied Fire text as support. The prejudicial consequence of Vincent's actions has been repeatedly demonstrated in attempts to draft claims specifically defining "short hairpins" in a manner that unambiguously distinguishes "short hairpins" from the "long hairpins" that the PTO (albeit improperly) now alleges are described by Fire.

45. In this regard, during prosecution of the '557 application, the examiner rejected Vincent's attempt to add a numerical limitation to the hairpin claims, noting that the attempt to add a specific numeric upper limit to such claims "is not consistent with the

specification and constitutes new matter where it is not disclosed nor made apparent by the disclosure of the specification that such a specific range was intended."

46. With respect to the short hairpin claims now pending in the '086 and '676 applications, Vincent's failure to properly supplement the '797 application with original disclosure specifically describing the short hairpin invention compounded the problem Vincent created by improperly relying on the copied Fire text. The continued harm these actions has caused is evident from the examiner's pending rejection of short hairpin claims in the '086 and '676 applications (filed from and claiming priority to the '797 application) as being anticipated by Fire.

47. Vincent's failure to properly distinguish Dr. Hannon's invention was further compounded by his failure to include additional disclosure regarding use of shRNA in mammalian cells that Dr. Hannon had available at the time that he filed the '797 application. This material, among other things, included data and other information Dr. Hannon provided in the article published as Genes & Development 16:948-958, a draft of which Dr. Hannon first sent to the journal on or about the '797 filing date.

48. In short, as a direct result of Vincent's and R&G's malpractice, the Hannon Applications have been unfairly prejudiced and compromised by the erroneous perception that the technology invented by Dr. Hannon is not sufficiently unique from what the Fire application describes to warrant a patent. Such a perception, created by Vincent's and R&G's misconduct, directly contributed to R&G's failure to obtain any allowed claims covering Dr. Hannon's invention.

49. Before, and increasingly throughout 2007, Mr. Maroney experienced frustration with a lack of communication from Vincent about the Hannon Applications, and in

particular about Vincent's understanding of why the PTO had withdrawn the '557 application from issue.

50. Dr. Vladimir Drozdoff is a Senior Licensing Associate and Patent Attorney for CSHL's Office of Technology Transfer. Dr. Drozdoff first began working at CSHL on February 11, 2008, with one of his first priorities being a review of Vincent's prosecution of the Hannon Applications.

51. Dr. Drozdoff first became aware of apparent copying of text from the Fire Specifications into the Hannon Applications upon reviewing the Office Action of September 4, 2007. In that Office Action, the Examiner noted the similarity of certain text to that in the Fire specification, in particular on both page 8 and page 10, that "*the disclosure of cell[s]/organisms of the instant specification at pages 21-22 is essentially verbatim of the disclosure of Fire et al. at column 8.*"

52. Dr. Drozdoff then compared the entire '557 specification with the entire Fire Patent, observing that the similarity extended beyond column 8 of the Fire Patent. Consequently, he conducted a further review of the Hannon Applications, which included a detailed comparison of the '739 application and the '097 application, to which the Hannon Applications claim priority, to the text of the Fire Patent and the published Fire application.

53. As a result of Dr. Drozdoff's investigation, the following facts were evident: (1) The "Summary of the Invention" of the '097 application contains approximately 11 pages of text that is almost identical to text found in the Fire application and in the Fire Patent; (2) this text is found in the Fire application in certain portions of 3 pages within the "Summary of Invention" and in certain portions of 13 pages within the "Detailed Description of the Invention"; (3) a substantial portion of this text was carried forward into the specification of the various

Hannon Applications; (4) none of the sections of the Hannon Applications where this text appears cite or reference the Fire application; and (5) the Fire application was first disclosed in the '557 prosecution in a November, 2004 filed IDS.

54. Mr. Maroney first became aware of apparent copying of text from the Fire Specifications into the Hannon Applications upon being advised of the same by Dr. Drozdoff on March 3, 2008. Before Dr. Drozdoff provided Mr. Maroney with this information on March 3, 2008, it had been his belief that the text of the '097 priority application and of the various Hannon Applications consisted of text drafted by Vincent along with text generated by the inventors, such as portions of draft manuscripts. This was the first time Mr. Maroney, or, to the best of his knowledge, anyone at CSHL, had been informed or had become aware that either the '097 priority application or any of the Hannon Applications contained numerous pages of text almost identical to text appearing in the Fire application.

55. Dr. Hannon first became aware of apparent copying of text from the Fire Specifications into the Hannon Applications upon being advised of the same by Dr. Drozdoff and Mr. Maroney on March 18, 2008.

56. To the extent that any papers were filed with the PTO, or any statements were made to the PTO, during the prosecution of the Hannon Applications, including any statements made to the PTO about the Fire Patent or the Fire application or any statements made about the Hannon Applications that involved sections that are the same as sections of the Fire Specifications, all such statements were made without any knowledge on the part of Dr. Drozdoff, Mr. Maroney, or Dr. Hannon, or, to the best of their knowledge, on the part of CSHL, that the specifications of the Hannon Applications contain text that is the same as, or very similar to, text from the Fire Specifications.

UNITED STATES DISTRICT COURT
EASTERN DISTRICT OF NEW YORK

COLD SPRING HARBOR LABORATORY,

Plaintiff,

-against-

ROPES & GRAY LLP and
MATTHEW P. VINCENT,

Defendants.

Civil Action No.:

COMPLAINT

(ST)

JURY TRIAL DEMANDED

Plaintiff, Cold Spring Harbor Laboratory ("CSHL"), by its attorneys, hereby alleges as follows:

PARTIES

1. CSHL is a New York education corporation chartered by the New York State Department of Education, with its principal place of business located in Cold Spring Harbor, New York.

2. Upon information and belief, defendant Ropes & Gray LLP ("R&G") is a Delaware limited liability partnership with its principal place of business in Boston, Massachusetts.

3. Defendant Matthew P. Vincent ("Vincent") was, until April 2009, a registered patent attorney and a partner in R&G's Intellectual Property group. Vincent received a Bachelors of Science degree in 1986 from Worcester Polytechnic Institute, a Ph.D. in Biochemical Sciences in 1991 from Tufts University School of Medicine, and a law degree in

57. Upon being made aware of the above facts, in accordance with CSHL's duty of candor and good faith in dealing with the PTO, and with CSHL's desire to maintain the highest levels of scientific integrity, Dr. Drozdoff and Mr. Maroney, acting on behalf of CSHL, took steps to further investigate and diligently rectify any impropriety that may have occurred in the prosecution of the Hannon Applications, as a result of the facts described above. These steps included meeting with Vincent and R&G partner James Haley to inform them of CSHL's findings, requesting R&G's full cooperation to provide the PTO with complete disclosure of these facts and in carrying out an entire, orderly transfer of responsibility for prosecution of the Hannon Applications from Vincent.

58. Accordingly, to understand what Vincent knew, Dr. Drozdoff and Mr. Maroney arranged to meet in New York City with Vincent and James Haley, a partner and patent attorney at R&G. During this meeting, which was held on April 1, 2008, Dr. Drozdoff and Mr. Maroney informed them of Dr. Drozdoff's findings regarding the similarity of certain text in the Hannon Applications and the Fire application and Fire Patent, and provided Vincent and Mr. Haley with a comparison of the '097 application to the Fire Specifications. Vincent acknowledged that in filing the '097 application, he was aware that portions thereof had been copied from the published Fire Specifications. At no time during this meeting did Vincent indicate that he had ever informed the PTO, CSHL or any of the co-inventors of this fact.

59. R&G refused to assure CSHL that it would unconditionally assist CSHL in providing the PTO with all relevant facts, instead imposing on CSHL the precondition that required CSHL to first sign a waiver essentially releasing R&G from any liability for misconduct. So that CSHL could carry out its first priority to provide the PTO with full disclosure, CSHL engaged Wilmer Cutler Pickering Hale and Dorr, LLP as new counsel.

60. As a result of their malpractice, R&G and Vincent failed to obtain allowance of any of the Hannon Applications and have caused CSHL to incur substantial, unnecessary and avoidable costs for their prosecution. Not only has CSHL been damaged in the form of hundreds of thousands of dollars in legal fees paid to R&G that would not have been necessary had Vincent and R&G met their duties of care, but CSHL, having been denied patents that it otherwise would have received but for R&G's and Vincent's negligence, has lost millions of dollars in potential licensing fees for the Hannon Applications.

Vincent's Termination by R&G and Subsequent Resignation from the Practice of Law

61. In late April 2009, R&G abruptly terminated Vincent's employment.

62. The purported basis for this termination is conduct by Vincent that was the subject of a pending State of Massachusetts disciplinary proceeding at the time of Vincent's termination, as described below.

63. On or about July 20, 2009, Vincent tendered his voluntary resignation from the practice of law, as a result of said investigation. Vincent's own affidavit in support thereof acknowledged the truth of the material facts upon which the disciplinary charges were based, including the following:

a. At some time prior to April 2002, Vincent formed a business entity known as "The IP Resource Company" to perform patent database searches;

b. Vincent did not inform his partners at R&G or his clients that he was the owner and operator of The IP Resource Company;

c. Beginning in approximately April 2002 and continuing through approximately September, 2008, Vincent prepared and submitted to R&G for payment sixty separate invoices from The IP Resource Company, each invoice relating to multiple patent matters;

d. The invoices that Vincent prepared stated, in summary form, that The IP Resource Company had performed research tasks on a total of approximately 3449 separate client matters and was entitled to payment of a total of $733,771.30 for those services. The invoices did not itemize costs, services rendered, dates on which services were rendered, or time spent.

e. Vincent approved each of the sixty invoices for payment and forwarded them to R&G's accounting department.

f. Relying on Vincent's approval, R&G paid the invoices and billed the appropriate clients for the service.

g. Vincent endorsed the checks for deposit and caused them to be deposited in an account for his personal use.

h. Vincent either never maintained or did not retain the underlying billing records for the invoices submitted by the IP Resource Company, and he cannot satisfactorily account for costs incurred and services rendered.

64. CSHL was among the clients of R&G who were victimized by Vincent's conduct described above. While serving as CSHL's primary patent prosecution counsel, R&G

billed and collected approximately $10,000 from CSHL in the name of work allegedly performed by IP Resource Company at the request, and/or with the approval, of R&G.

FIRST CLAIM
(Legal Malpractice)

65. CSHL repeats and realleges the allegations made in paragraphs 1 through 64 above.

66. Vincent and R&G acted as attorneys for CSHL from in or around 2001 through September 2008. During this time frame, Vincent and R&G owed CSHL a duty of care.

67. During this time frame, Vincent and R&G were the only attorneys acting on behalf of CSHL in the patent prosecution of the Hannon Applications.

68. In taking the actions, and omitting to act, as described in Paragraphs 22-60 above, Mr. Vincent's and R&G's actions were negligent and fell below the applicable professional standards for representing an entity in CSHL's position in pursuing patents such as the Hannon Applications.

69. But for Vincent's and R&G's failures to satisfy their duties of care to CSHL, it would have been able to obtain allowance of claims covering Dr. Hannon's inventions.

70. As a direct and proximate result of Vincent's and R&G's negligence, CSHL has been severely damaged. CSHL has spent hundreds of thousands of dollars on legal fees that would not have been necessary had Vincent and R&G met their duties of care. These fees include substantial additional costs that CSHL has incurred resulting from its transfer of prosecution to new counsel and in legal work that new counsel has conducted in an attempt to address the harm caused by R&G's misconduct to the Hannon Applications, as well as fees paid to R&G that would not have been necessary had Vincent and R&G satisfied their duties of care.

CSHL will establish the exact amount of this category of damages at trial, but expects it to be no less than $1,000,000.

71. In addition, CSHL has lost opportunities for licensing Dr. Hannon's technology, including at minimum, lost opportunities resulting from the loss of patent term caused directly by Vincent's and R&G's malpractice. The resulting losses include lost opportunities for commercial user licenses, allowing commercial use of shRNA technology, as well as lost royalty income on research reagent sales. The exact amount of such damages will be established at trial. However, CSHL expects that the annual amount of such licensing opportunities it has lost as a direct and proximate result of Vincent's and R&G's malpractice to be at least $22,500,000 for lost commercial user license income and $9,000,000 for lost royalty income, should CSHL ultimately obtain the patent such that only five years of patent term are lost, to $57,500,000 for lost commercial user license income and $19,000,000 for lost royalty income, should CSHL be denied the patent such that fifteen years of patent term are lost.

72. In addition, as a direct and proximate result of their negligence, Vincent's and R&G's failure to obtain issuance of claims to CSHL's shRNA technology has precluded CSHL from pursuing opportunities to further commercialize this technology through a start-up company. The exact amount of such damages will be established at trial. However, CSHL expects that the start-up income it has lost as a direct and proximate result of Vincent's and R&G's malpractice to be at least $5,000,000. In sum total, depending on the number of years of term lost, CSHL expects its total damages as a direct and proximate result of Vincent's and R&G's malpractice to be at least $37,500,000 to $82,500,000.

SECOND CLAIM
(Breach of Fiduciary Duty)

73. CSHL repeats and realleges the allegations made in paragraphs 1 through 72 above.

74. As attorneys for CSHL, R&G and Vincent owed CSHL a fiduciary duty.

75. In taking the actions, and omitting to act, as described in Paragraphs 22-60 above, Mr. Vincent's and R&G's actions were in breach of their fiduciary duties to CSHL.

76. As a direct and proximate result of R&G's and Vincent's breaches of their fiduciary duties to CSHL, as described above, CSHL has been damaged in an amount to be determined at trial.

77. Specifically, CSHL has been damaged in the form of: (i) additional attorneys' fees that it has been forced to expend as a result of R&G's and Vincent's wrongful acts and omissions, which is estimated to be no less than $500,000; (ii) lost licensing opportunities for the Hannon technology, which is estimated to be worth no less than $36,500,000 to $81,500,000; and (iii) disgorgement of all attorneys' fees paid by CSHL to R&G since 2001, which is estimated to be no less than $1,400,000.

THIRD CLAIM
(Fraud and Fraudulent Concealment)

78. CSHL repeats and realleges the allegations made in paragraphs 1 through 77 above.

79. For more than eight years, while acting as primary patent prosecution counsel for CSHL, by intentionally and repeatedly concealing Vincent's copying of Fire, Vincent and R&G committed a fraud upon CSHL.

80. Vincent and R&G failed to inform CSHL of the copying of Fire, and failed to inform the PTO of the copying of Fire, despite their professional obligation to make

such disclosure. This withholding of material information was intentionally done by Vincent and R&G for the purpose of, and had the effect of, inducing CSHL to continue using Vincent and R&G as its primary patent counsel, and to reasonably rely on their advice in the mistaken belief that they had undertaken to provide professional advice in CSHL's best interests and had done nothing to stain CSHL's reputation or credibility with the PTO, or prejudice its patent applications.

81. On this point, shortly before filing the March 9, 2007 Amendment in the '557 application, Vincent went with Dr. Hannon and Mr. Maroney to meet with the examiner, essentially over the examiner's pending rejection of claims as being anticipated by Fire. Incredibly, Vincent still never brought to their attention the copying and resulting problems that he created in distinguishing Dr. Hannon's work from Fire.

82. Had Vincent been forthright about his copying of Fire anytime during the course of the prosecution of the Hannon Applications, CSHL would never have kept Vincent and R&G as its patent counsel. By intentionally concealing what actually had transpired, Vincent and R&G intentionally induced CSHL to continue using Vincent and R&G for a majority of its patent work.

83. Vincent and R&G, as attorneys for CSHL, had a duty to disclose their copying of Fire and the prejudice to the Hannon Applications that their continued reliance on the copied text had caused, as described above, which was material information.

84. As a result of this fraudulent concealment and CSHL's reasonable reliance on the deceptive statements made by Vincent and R&G (via the filed Hannon Applications) that failed to disclose the copying of Fire, CSHL has been damaged in an amount to be determined at trial, including punitive damages.

85. Vincent committed a separate fraud upon CSHL through his tendering, through R&G, of invoices from The IP Resource Company totaling at least $9587.45, all of which were paid by CSHL, notwithstanding that, upon information and belief, Vincent could not substantiate any work performed by that company for the benefit of CSHL. The statements of work performed set forth on these invoices constituted material misrepresentations.

86. Vincent tendered these invoices to CSHL, through R&G, knowing that they were false, with the intent that CSHL would rely on the statements contained in those invoices, and the fact that they were being tendered to CSHL by R&G, in making payment thereon.

87. CSHL reasonably relied on the statements of work performed contained in the invoices of The IP Resource Company invoices forwarded for payment by R&G.

88. In making payment on these fraudulent invoices, CSHL suffered damages in an amount to be determined at trial, plus punitive damages.

JURY DEMAND

Pursuant to Rule 38 of the Federal Rules of Civil Procedure, CSHL demands a trial by jury of all of its claims.

PRAYER FOR RELIEF

Wherefore, CSHL prays for judgment against R&G and Vincent as follows:

a. Granting R&G and Vincent judgment on its Claims in a sum to be determined at trial, which is expected to be no less than $37,500,000 to $82,500,000 plus punitive damages;

b. Awarding CSHL attorneys fees and costs; and

c. Awarding CSHL such other and further relief as this Court deems just and proper.

Dated: February 16, 2010
Garden City, New York

Chad E. Ziegler [CZ5717] [Pro Hac Vice-pending]
Peter I. Bernstein [PB3549]
Scully, Scott, Murphy & Presser, P.C.
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Garden City, New York 11530
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UNITED STATES DISTRICT COURT
EASTERN DISTRICT OF NEW YORK

COLD SPRING HARBOR LABORATORY,

Plaintiff,

-against-

ROPES & GRAY LLP and
MATTHEW P. VINCENT,

Defendants.

Civil Action No.:

COMPLAINT

(SI)

JURY TRIAL DEMANDED

Plaintiff, Cold Spring Harbor Laboratory ("CSHL"), by its attorneys, hereby alleges as follows:

PARTIES

1. CSHL is a New York education corporation chartered by the New York State Department of Education, with its principal place of business located in Cold Spring Harbor, New York.

2. Upon information and belief, defendant Ropes & Gray LLP ("R&G") is a Delaware limited liability partnership with its principal place of business in Boston, Massachusetts.

3. Defendant Matthew P. Vincent ("Vincent") was, until April 2009, a registered patent attorney and a partner in R&G's Intellectual Property group. Vincent received a Bachelors of Science degree in 1986 from Worcester Polytechnic Institute, a Ph.D. in Biochemical Sciences in 1991 from Tufts University School of Medicine, and a law degree in

1996 from Suffolk University Law School in Boston. Upon information and belief, Vincent is a resident of the State of Massachusetts.

4. As discussed in greater detail below, on or about July 20, 2009, Vincent filed his resignation from the practice of law with the Office of the Bar Counsel for the State of Massachusetts. Vincent's resignation resulted from an investigation into his misconduct that was the subject of a disciplinary investigation revolving around his having, for more than six years, under cover of a separate company which he formed, billed and collected from R&G's clients more than $700,000 for work that could not be substantiated or verified, purportedly without R&G's knowledge that he owned said company. In late April 2009, R&G terminated Vincent's employment, purportedly because of the misconduct described above.

JURISDICTION AND VENUE

5. This Court has subject matter jurisdiction over this action pursuant to 28 U.S.C. § 1338(a).

6. Venue is appropriate in this judicial district pursuant to 28 U.S.C. § 1391(b)(1) in that R&G is subject to personal jurisdiction in this district and, thus, is a resident of this district, and pursuant to § 1391(b)(2) in that a substantial part of the events or omissions giving rise to CSHL's claims occurred in, and a substantial part of the property that is the subject of the action is situated in, this district.

SUMMARY OF CLAIMS

7. This case involves the negligence of R&G in prosecuting a series of patent applications on behalf of CSHL. These patent applications are directed to inventions made by Dr. Gregory Hannon, a Professor and Howard Hughes Medical Institute Investigator at CSHL, and his colleagues at CSHL (collectively, "Dr. Hannon"), which exploit a cellular mechanism

1996 from Suffolk University Law School in Boston. Upon information and belief, Vincent is a resident of the State of Massachusetts.

4. As discussed in greater detail below, on or about July 20, 2009, Vincent filed his resignation from the practice of law with the Office of the Bar Counsel for the State of Massachusetts. Vincent's resignation resulted from an investigation into his misconduct that was the subject of a disciplinary investigation revolving around his having, for more than six years, under cover of a separate company which he formed, billed and collected from R&G's clients more than $700,000 for work that could not be substantiated or verified, purportedly without R&G's knowledge that he owned said company. In late April 2009, R&G terminated Vincent's employment, purportedly because of the misconduct described above.

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SUMMARY OF CLAIMS

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called RNA interference ("RNAi"). Generally, RNAi refers to the process by which double stranded RNA functions to help regulate when genes are turned off and on in the cell. Through his work at CSHL, Dr. Hannon developed novel methods and technologies to exploit RNAi as a research tool in mammalian cells, which Dr. Hannon achieved by engineering RNA molecules called short hairpin RNAs (shRNAs). Among its many applications, the shRNA technology Dr. Hannon invented gives researchers the ability to specifically turn off expression of virtually any target gene or combination of target genes in a mammalian cell. This valuable technology provides an efficient, effective, widely applicable alternative to more expensive and laborious methods for research and drug development. Today, shRNA has become a fundamental tool in biomedical research for studying what genes do in cells, what goes wrong in diseases such as cancer and for identifying drug targets.

8. In its prosecution of patent applications intended to cover Dr. Hannon's shRNA inventions (the "Hannon Applications"), R&G, and in particular, Vincent, the R&G attorney responsible for prosecution of these patents, committed malpractice through their failure to conduct the prosecution according to a reasonable standard of care, with the result that their conduct has both delayed and prejudiced CSHL's efforts to obtain patent claims covering Dr. Hannon's inventions in several ways, as summarized below.

9. When Vincent drafted the three earliest non-provisional Hannon Applications (U.S. patent applications nos. 09/858,862, filed May 16, 2001, 09/866,557, filed March 24, 2001 and international patent application PCT/US01/08345, filed March 16, 2001), rather than providing an original, complete description of Dr. Hannon's work, Vincent instead relied upon copying extensive portions of text -- essentially verbatim -- from a prior patent application (WO/99/32619) published by a team led by another researcher in the RNAi field, Dr.

Andrew Fire (collectively, "Fire"), to at least, in part, describe Dr. Hannon's inventions. About one half of the "Detailed Description of Certain Preferred Embodiments" found in the three earliest filed Hannon Applications consists of text copied from the Fire application. As described below, Vincent continued to rely upon this text to describe Dr. Hannon's inventions, and in particular, the shRNA technology that is the subject of the pending Hannon Applications. By relying extensively on the copied text, Vincent failed to fully describe and distinguish Dr. Hannon's inventions from the different technology invented by Fire.

10. During the course of prosecution, Vincent and R&G filed numerous follow-up continuation and continuation-in-part ("CIP") applications, allowing several opportunities to properly re-draft the Hannon Applications in such a way that relied on an original description of Dr. Hannon's own work to accurately describe the shRNA technology that Dr. Hannon invented. Instead, R&G and Vincent continued to rely upon the text copied from the Fire application, which falsely implied that Dr. Hannon's shRNA technology was either something that Fire invented or was suggested by the Fire application.

11. R&G and Vincent compounded the prejudice resulting from their continued use of the copied Fire text by failing to properly supplement the foregoing CIP applications with Dr. Hannon's ongoing work in a timely fashion. Furthermore, neither R&G nor Vincent ever brought the fact of this copying of the Fire text, and the potential prejudice resulting from that copying, to the attention of Dr. Hannon and CSHL. Despite being aware of how their negligent conduct had compromised the Hannon Applications, R&G and Vincent continued to prosecute the applications while hiding this fact from Dr. Hannon and CSHL. Vincent and R&G further compounded their negligence by their failure to properly attribute the copied text to Fire, effectively continuing to misrepresent that disclosure as being part of Dr.

Hannon's work, and by years of delay in disclosing the Fire application to the United States Patent & Trademark Office (the "PTO"). Significantly, all this deprived Dr. Hannon and CSHL of any opportunity to mitigate the harm caused by Vincent and R&G's negligent prosecution, including the opportunity to appropriately re-draft the specification in a timely fashion to minimize any potential loss of priority.

12. It was the PTO that first noted the similarity of certain text in the Hannon Applications to that in the Fire application, a fact which ultimately brought R&G's and Vincent's misconduct to CSHL's attention. That fact, however, came to CSHL's attention only after Vincent and R&G had already caused irreparable harm through their negligent prosecution of the Hannon Applications. By the time the PTO eventually cited the Fire application as prior art against Dr. Hannon's invention, the Hannon Applications had been unfairly prejudiced by the erroneous perception that the technology invented by Dr. Hannon is not sufficiently unique from what the Fire application describes to warrant a patent.

STATEMENT OF FACTS

Background and Description of Relevant Technologies

13. CSHL is a private, not-for-profit research and education institution at the forefront of efforts in molecular biology and genetics to generate knowledge that will yield better diagnostics and treatments for cancer, neurological diseases and other major causes of human suffering.

14. Home to eight Nobelists, CSHL was founded in 1890 as one of the first institutions to specialize in genetics research and subsequently has played a central role in the seminal field of molecular biology. At CSHL in 1953, James D. Watson presented his first public lecture on his and Francis Crick's discovery of the double-helical structure of DNA, for

which each later won a Nobel Prize. As Director and then President of the Laboratory from 1968 to 2003, Watson was instrumental in developing CSHL into one of the world's most influential cancer research centers.

15. Today, more than 400 scientists at CSHL pioneer the frontiers of biomedical research. A designated Center of the National Cancer Institute, CSHL has broken new ground in the study of cancer genetics. It has also taken a leading role in efforts to understand what causes neurodevelopmental and neurodegenerative illnesses such as autism, schizophrenia, and Alzheimer's and Parkinson's diseases, and is a global leader in plant genetics and in the emerging discipline of quantitative biology.

16. Each year 8,000 of the world's leading life scientists are drawn to the campus for CSHL's legendary Meetings and Courses program, where new research is discussed and debated. The CSHL Press publishes textbooks and research journals that are among the most highly cited in their fields. CSHL also has created the DNA Learning Center, the nation's first science center dedicated to public genetics education. Its hands-on programs have reached 325,000 middle and high school students, teachers, and families since 1988, and its award-winning website millions more.

17. With regard to the Hannon Applications, of particular importance are the methods and technologies Dr. Hannon invented to use shRNAs in human and other mammalian cells. Since Dr. Hannon's invention, use of shRNA for gene silencing and regulation has become a valuable and widely adopted technology, which is used today in many different fields of medical and pharmaceutical research.

18. In 2002, Dr. Hannon's research on RNA interference was recognized by Science magazine as the Breakthrough of the Year and in 2005 by Esquire as a Breakthrough of

the Decade. Recognized as one of the world's most accomplished scientists, Dr. Hannon has received numerous awards, including appointment as a Pew Scholar in the Biomedical Sciences and as a Rita Allen Foundation Scholar. In 2003, he received the U.S. Army Breast Cancer Research Program's Innovator Award; in 2005 the American Association for Cancer Research's Award for Outstanding Achievement in Cancer Research and in 2007 he received the National Academy of Sciences Award for Molecular Biology and The Memorial Sloan-Kettering Cancer Center's Paul Marks Prize for Cancer Research. He assumed his current position in 2005 as a Howard Hughes Medical Institute Professor and continues to explore the mechanisms and regulation of RNA interference as well as its applications to cancer research.

19. CSHL is the assignee of the entire right, title, and interest in the Hannon Applications, which collectively refer to U.S. patent application numbers 09/858,862 filed May 16, 2001 ("the '862 application"), 09/866,557, filed March 24, 2001 ("the '557 application), 10/055,797 filed January 22, 2002 ("the '797 application"), 10/350,798 filed January 24, 2003, 10/997,086, filed November 23, 2004, 11/791,554 filed May 23, 2007, 11/894,676 filed August 20, 2007, 12/152,655 filed May 15, 2008, 12/152,837 filed May 16, 2008 and international patent applications PCT/US01/08435 filed March 16, 2001 ("the '435 PCT application"), PCT/US03/01963 filed January 22, 2003, and PCT/US05/42488 filed November 23, 2005, including all foreign patent applications filed therefrom. Certain of the Hannon Applications claim a benefit of priority to U.S. provisional applications 60/189,739 filed March 16, 2000 ("the '739 application"), and 60/243,097 filed October 24, 2000 ("the '097 application).

20. International Patent Application PCT/US98/27233, which was published on July 1, 1999 with International Publication Number WO/99/32619 (the "Fire application"), describes certain work conducted by Fire relating to his discovery that long double stranded

RNA molecules could specifically silence gene expression in invertebrate cells. Fire referred generally to this cellular process as RNA interference, or RNAi.

21. Fire received U.S. Patent No. 6,506,559 for his RNAi technology, which issued on January 14, 2003 (the "Fire Patent"). The essentially identical written disclosures of the Fire application and the Fire Patent are referred to collectively hereinafter as the "Fire Specification."

Facts Relating to Malpractice

22. From in or around 2001 until late 2008, R&G acted as principal outside patent prosecution counsel for CSHL.

23. Vincent was the R&G attorney primarily involved in the drafting and prosecution of the all of the Hannon Applications, as well as the '739 application and the '097 application, to which certain of the Hannon Applications claim priority. To date, CSHL has paid R&G approximately $420,000 in legal fees and disbursements that R&G has billed for its prosecution of the patent applications related to Dr. Hannon's shRNA technologies, and approximately $1,400,000 in fees and disbursements that R&G has billed for its prosecution of other applications.

24. The '097 application includes about 11 pages of text that Vincent copied essentially verbatim, without citation or attribution, from the published Fire application. Attached as Exhibit A is a "marked-up" version of the '097 application, in which text that is the same as text in the Fire Specification is highlighted. Vincent specifically carried over at least some portion of the copied Fire text found in the '097 application into all of the subsequently filed Hannon Applications.

25. Despite the fact that Vincent was fully aware of the Fire application as early as October 2000, when he first copied text from that application into the '097 application, R&G did not cite the Fire application in any papers filed on behalf of CSHL until the Supplemental Information Disclosure Statement of November 26, 2004. And, R&G did not cite the Fire Patent in any papers filed on behalf of CSHL until the Supplemental Information Disclosure Statement of January 7, 2005.

26. The '435 PCT application, filed March 16, 2001, the '862 application filed May 16, 2001, and the '557 application filed May 24, 2001 are directed generally to the initial methods and technologies Dr. Hannon developed relating to use of RNA interference in mammalian and other cells, including use of hairpin RNAs to regulate target genes. In particular, the filed '557 application included claims (20-25) directed to use of hairpin RNAs to inhibit gene expression and expression of such hairpin RNAs in cells of a transgenic non-human mammal.

27. About one half of the "Detailed Description of Certain Preferred Embodiments" (hereinafter, the "Detailed Description") found in the three earliest filed non-provisional Hannon Applications consists of text copied from the Fire application. Vincent's failure to provide an adequate description of Dr. Hannon's technology in these applications seriously compromised the ability of these applications, in particular the '557 application, to serve as priority support for Dr. Hannon's patent claims. This fact has deprived CSHL of the opportunity to obtain allowance of claims covering Dr. Hannon's inventions entitled to the respective filing dates of these applications, based on the support from these applications.

28. Vincent repeatedly effectuated this copying of Fire, notwithstanding that he knew from the outset that the Hannon Applications needed to be distinguished from Fire.

29. In this regard, the now pending claims of the Hannon '086 application are directed to one particularly valuable aspect of the technology Dr. Hannon developed, methods that allow one to stably suppress gene expression in mammalian cells using RNA interference. Among other things, this valuable technology provides an efficient, effective and widely applicable alternative to more expensive and laborious methods for biomedical research and drug development. Dr. Hannon's shRNA methods represented a considerable advance over the prior art, including the Fire patent, which failed to provide any solution for how to use RNA interference in mammals, without killing the treated cells through the so-called "interferon" or "protein kinase (PK) response".

30. Vincent first added claims directed to use of hairpin RNAs to inhibit gene expression and expression of such hairpin RNAs in cells of a transgenic non-human mammal in the '557 application, which was filed May 24, 2001 as a continuation-in-part ("CIP") of the '435 PCT application. Both from the standpoint of meeting his duty of care and scientifically, in adding these new claims, Vincent had an affirmative duty to amend the specification with original text accurately describing these additional claimed inventions and distinguish these from the Fire application. However, instead of properly amending the Detailed Description, Vincent again relied on the same text he had previously copied from the Fire application as support for these new claims, knowing full well that text copied directly from Fire could not serve to distinguish the newly claimed subject matter from Fire.

31. During 2001, Vincent regularly communicated with Dr. Hannon regarding the Hannon Applications Vincent was then prosecuting. In filing the '557 application on May 24, 2001, and also in filing the '797 application in January 22, 2002, Vincent either knew or should have known the relevance and potential prejudice of continuing to rely on extensive

passages of text copied from the Fire application. He should have told Dr. Hannon then what he had done. However, before filing the '557 application, and even the '797 application, Vincent failed to inform Dr. Hannon and CSHL (either directly or through Dr. Hannon) of this copying of Fire and Vincent's continued use of this copied text in the Detailed Description as support for the filed claims. His failure to do so deprived Dr. Hannon and CSHL of the timely opportunity to amend the specification to properly describe and distinguish Dr. Hannon's technology from the different methods Fire described.

32. As corroborated by laboratory and other records, by the time Vincent had filed the '557 application, Dr. Hannon had already conceived of the short hairpin methods that are the subject of the now pending Hannon claims. Had Vincent in May 2001 informed CSHL and Dr. Hannon of his conduct, such information would have identified an urgent need to amend the '557 application to distinguish Dr. Hannon's hairpin technology, including in particular the use of short hairpins, from Fire's altogether different disclosure. Such amendment would necessarily have included adding original disclosure describing Dr. Hannon's short hairpin invention.

33. Vincent's failure to inform CSHL and Dr. Hannon of his conduct resulted in an entirely unnecessary and prejudicial delay in adding specific disclosure about the short hairpin invention to the Hannon Applications. To the extent Vincent eventually did so, this happened eight months later with the filing of the '797 CIP application on January 22, 2002. Even then, instead of revising the Detailed Description to provide an accurate description of the short hairpin technology, Vincent continued to improperly rely on the text he had copied from the Fire application, fully knowing that this text was directed to an entirely different invention. Had Vincent been forthright in May of 2001 about his copying of the Fire text, Vincent would

called RNA interference ("RNAi"). Generally, RNAi refers to the process by which double stranded RNA functions to help regulate when genes are turned off and on in the cell. Through his work at CSHL, Dr. Hannon developed novel methods and technologies to exploit RNAi as a research tool in mammalian cells, which Dr. Hannon achieved by engineering RNA molecules called short hairpin RNAs (shRNAs). Among its many applications, the shRNA technology Dr. Hannon invented gives researchers the ability to specifically turn off expression of virtually any target gene or combination of target genes in a mammalian cell. This valuable technology provides an efficient, effective, widely applicable alternative to more expensive and laborious methods for research and drug development. Today, shRNA has become a fundamental tool in biomedical research for studying what genes do in cells, what goes wrong in diseases such as cancer and for identifying drug targets.

8. In its prosecution of patent applications intended to cover Dr. Hannon's shRNA inventions (the "Hannon Applications"), R&G, and in particular, Vincent, the R&G attorney responsible for prosecution of these patents, committed malpractice through their failure to conduct the prosecution according to a reasonable standard of care, with the result that their conduct has both delayed and prejudiced CSHL's efforts to obtain patent claims covering Dr. Hannon's inventions in several ways, as summarized below.

9. When Vincent drafted the three earliest non-provisional Hannon Applications (U.S. patent applications nos. 09/858,862, filed May 16, 2001, 09/866,557, filed March 24, 2001 and international patent application PCT/US01/08345, filed March 16, 2001), rather than providing an original, complete description of Dr. Hannon's work, Vincent instead relied upon copying extensive portions of text -- essentially verbatim -- from a prior patent application (WO/99/32619) published by a team led by another researcher in the RNAi field, Dr.

have no doubt been apprised then (if he was not already aware) of Dr. Hannon's work relating to the short hairpin invention. Instead, by waiting until January 2002 to get reference to short hairpins into Dr. Hannon's applications, Vincent caused a potential crucial loss of priority from May 2001.

34. In short, even after the filing of the '557 application, Vincent and R&G failed to comply with a reasonable standard of care in the subsequent prosecution of these applications and the filing of subsequent applications in the PTO based on these parent applications. Vincent and R&G never brought the fact of Vincent's copying of the Fire text, and the potential prejudice resulting from that copying, to the attention of CSHL. Despite being aware of how his conduct had compromised the Hannon Applications, Vincent continued to prosecute them while hiding this fact from CSHL. Significantly, this deprived CSHL of any opportunity to address the issues the copying raised early in prosecution, when the applications could have been re-drafted in a timely fashion to minimize any potential loss of priority and minimize the harm to CSHL.

35. Further compounding the harm Vincent had caused, in prosecuting these early applications, Vincent's and R&G's improperly relied on and misrepresented the copied Fire text as describing the technology Dr. Hannon invented. In effect, their actions erroneously implied that Dr. Hannon's technology was previously invented or described by Fire, which it was not. Through their failure to properly attribute the copied text to Fire, and years of delay in even bringing the Fire application and Fire Patent to the attention of the PTO, Vincent and R&G further compounded their malpractice by effectively continuing to misrepresent that disclosure as being part of Dr. Hannon's work.

36. For example, these misrepresentations include statements made during prosecution of the '557 application. In the office action dated April 21, 2005 rejecting all pending claims, the PTO Examiner argued that the specification failed to teach introducing an expression vector encoding a hairpin RNA into mammalian cells. In response (Reply and Amendment filed August 11, 2005), R&G argued that the application described the use of expression systems that are intended to produce hairpin RNAs upon being transcribed in cells. In support, R&G repeatedly pointed to various sections of text copied from the Fire application. To support its position, R&G filed a Rule 132 Expert Declaration (Declaration under 35 U.S.C. §1.132 of Frank McKeon dated July 29, 2005), which cited repeatedly to sections of the copied Fire text as evidence that the technology invented by Dr. Hannon and described in the '557 application was directed to use of expression systems intended to produce hairpin RNAs upon being transcribed in cells.

37. As stated above, instead of properly re-drafting the Hannon Applications via the numerous CIP applications, in a way that relied upon an original description of Dr. Hannon's work, Vincent and R&G continued to rely on text copied from Fire despite the fact that this risked the false implication that Dr. Hannon's shRNA technology was either something that Fire invented or was suggested by the Fire application.

38. Notably, on August 11, 2005, the PTO issued a Notice of Allowance for the pending '557 application claims. In explaining his reasons for allowance, the Examiner stated on page 3:

> The declarations under 37 CFR 1.132 filed August 11, 2005 are sufficient to overcome the rejections of claims...based upon new matter under 35 USC 112 first paragraph and lack of enablement under 35 USC 112 first paragraph. Specifically, the declaration of Frank Mckeon establishes that the double stranded RNA construct

> of the patent application encompass hairpin RNA comprising the features claimed therein. The declaration provides evidence by specific examples in the instant application.

39. On April 6, 2006, however, the PTO withdrew the '557 application from issue to reconsider its decision. On September 6, 2006, the Examiner rejected all pending claims as anticipated by the Fire Patent.

40. The prejudice to the Hannon Applications caused by Vincent's original attempt to describe the Hannon inventions by copying text from Fire application was illustrated by R&G's subsequent failed efforts to overcome the Fire Patent as prior art against the '557 application. In the Amendment filed March 9, 2007, Vincent argued that in contrast to the disclosure of the '557 application, the Fire Patent failed to provide any particular guidance that would have led one to envisage the claimed methods directed to using an expression vector encoding a hairpin RNA to attenuate gene expression specifically in mammalian cells.

41. In fact, before filing the March 9, 2007 Amendment, Vincent conducted an in-person interview in February with Examiner McGarry and Supervisory Examiner Schultz at the PTO, specifically to discuss the Examiner McGarry's rejection of the pending claims as anticipated by the Fire Patent. Notably, Vincent brought with him to the interview both Dr. Hannon and John Maroney, the Vice President, Legal Counsel, and Director of CSHL's Office of Technology Transfer. During this interview, Vincent provided the Examiners with a preview of the argument he planned to present in the March 9, 2007 Amendment. Despite the fact that his planned arguments relied on text copied from the Fire specification, Vincent never disclosed this fact to Examiners McGarry and Schultz, Dr. Hannon or Mr. Maroney.

42. In the Office Action dated September 4, 2007, the PTO rejected Vincent's argument that only the '557 specification provided such guidance. Referring specifically to the text that Vincent had copied from Fire, the PTO noted that "in fact the disclosure of cell/organisms of the instant specification at pages 21-22 is essentially verbatim of the disclosure of Fire et al at column 8," and that "it is unclear how applicant claimed invention differs from what has been disclosed by the prior art."

43. The '797 application was filed as a continuation-in-part of the '435 PCT application and among other things, incorporated additional disclosures from Dr. Hannon relating to the use of the shRNA technology for regulating gene expression in mammalian cells. This added material was directed to an entirely different invention from the technology described in the Fire application. Despite that fact, Vincent retained the copied Fire text in the Detailed Description, fully knowing that the copied Fire text described a different invention.

44. In copying Fire again in filing the '797 application, Vincent furthered his malpractice by again failing to make any reasonable effort to amend the specification, in either the summary or detailed description of the invention, to accurately support claims directed to use of short hairpins in mammalian cells. Instead, Vincent continued to rely on the extensively copied Fire text as support. The prejudicial consequence of Vincent's actions has been repeatedly demonstrated in attempts to draft claims specifically defining "short hairpins" in a manner that unambiguously distinguishes "short hairpins" from the "long hairpins" that the PTO (albeit improperly) now alleges are described by Fire.

45. In this regard, during prosecution of the '557 application, the examiner rejected Vincent's attempt to add a numerical limitation to the hairpin claims, noting that the attempt to add a specific numeric upper limit to such claims "is not consistent with the

specification and constitutes new matter where it is not disclosed nor made apparent by the disclosure of the specification that such a specific range was intended."

46. With respect to the short hairpin claims now pending in the '086 and '676 applications, Vincent's failure to properly supplement the '797 application with original disclosure specifically describing the short hairpin invention compounded the problem Vincent created by improperly relying on the copied Fire text. The continued harm these actions has caused is evident from the examiner's pending rejection of short hairpin claims in the '086 and '676 applications (filed from and claiming priority to the '797 application) as being anticipated by Fire.

47. Vincent's failure to properly distinguish Dr. Hannon's invention was further compounded by his failure to include additional disclosure regarding use of shRNA in mammalian cells that Dr. Hannon had available at the time that he filed the '797 application. This material, among other things, included data and other information Dr. Hannon provided in the article published as Genes & Development 16:948-958, a draft of which Dr. Hannon first sent to the journal on or about the '797 filing date.

48. In short, as a direct result of Vincent's and R&G's malpractice, the Hannon Applications have been unfairly prejudiced and compromised by the erroneous perception that the technology invented by Dr. Hannon is not sufficiently unique from what the Fire application describes to warrant a patent. Such a perception, created by Vincent's and R&G's misconduct, directly contributed to R&G's failure to obtain any allowed claims covering Dr. Hannon's invention.

49. Before, and increasingly throughout 2007, Mr. Maroney experienced frustration with a lack of communication from Vincent about the Hannon Applications, and in

particular about Vincent's understanding of why the PTO had withdrawn the '557 application from issue.

50. Dr. Vladimir Drozdoff is a Senior Licensing Associate and Patent Attorney for CSHL's Office of Technology Transfer. Dr. Drozdoff first began working at CSHL on February 11, 2008, with one of his first priorities being a review of Vincent's prosecution of the Hannon Applications.

51. Dr. Drozdoff first became aware of apparent copying of text from the Fire Specifications into the Hannon Applications upon reviewing the Office Action of September 4, 2007. In that Office Action, the Examiner noted the similarity of certain text to that in the Fire specification, in particular on both page 8 and page 10, that "*the disclosure of cell[s]/organisms of the instant specification at pages 21-22 is essentially verbatim of the disclosure of Fire et al. at column 8.*"

52. Dr. Drozdoff then compared the entire '557 specification with the entire Fire Patent, observing that the similarity extended beyond column 8 of the Fire Patent. Consequently, he conducted a further review of the Hannon Applications, which included a detailed comparison of the '739 application and the '097 application, to which the Hannon Applications claim priority, to the text of the Fire Patent and the published Fire application.

53. As a result of Dr. Drozdoff's investigation, the following facts were evident: (1) The "Summary of the Invention" of the '097 application contains approximately 11 pages of text that is almost identical to text found in the Fire application and in the Fire Patent; (2) this text is found in the Fire application in certain portions of 3 pages within the "Summary of Invention" and in certain portions of 13 pages within the "Detailed Description of the Invention"; (3) a substantial portion of this text was carried forward into the specification of the various

Hannon Applications; (4) none of the sections of the Hannon Applications where this text appears cite or reference the Fire application; and (5) the Fire application was first disclosed in the '557 prosecution in a November, 2004 filed IDS.

54. Mr. Maroney first became aware of apparent copying of text from the Fire Specifications into the Hannon Applications upon being advised of the same by Dr. Drozdoff on March 3, 2008. Before Dr. Drozdoff provided Mr. Maroney with this information on March 3, 2008, it had been his belief that the text of the '097 priority application and of the various Hannon Applications consisted of text drafted by Vincent along with text generated by the inventors, such as portions of draft manuscripts. This was the first time Mr. Maroney, or, to the best of his knowledge, anyone at CSHL, had been informed or had become aware that either the '097 priority application or any of the Hannon Applications contained numerous pages of text almost identical to text appearing in the Fire application.

55. Dr. Hannon first became aware of apparent copying of text from the Fire Specifications into the Hannon Applications upon being advised of the same by Dr. Drozdoff and Mr. Maroney on March 18, 2008.

56. To the extent that any papers were filed with the PTO, or any statements were made to the PTO, during the prosecution of the Hannon Applications, including any statements made to the PTO about the Fire Patent or the Fire application or any statements made about the Hannon Applications that involved sections that are the same as sections of the Fire Specifications, all such statements were made without any knowledge on the part of Dr. Drozdoff, Mr. Maroney, or Dr. Hannon, or, to the best of their knowledge, on the part of CSHL, that the specifications of the Hannon Applications contain text that is the same as, or very similar to, text from the Fire Specifications.

57. Upon being made aware of the above facts, in accordance with CSHL's duty of candor and good faith in dealing with the PTO, and with CSHL's desire to maintain the highest levels of scientific integrity, Dr. Drozdoff and Mr. Maroney, acting on behalf of CSHL, took steps to further investigate and diligently rectify any impropriety that may have occurred in the prosecution of the Hannon Applications, as a result of the facts described above. These steps included meeting with Vincent and R&G partner James Haley to inform them of CSHL's findings, requesting R&G's full cooperation to provide the PTO with complete disclosure of these facts and in carrying out an entire, orderly transfer of responsibility for prosecution of the Hannon Applications from Vincent.

58. Accordingly, to understand what Vincent knew, Dr. Drozdoff and Mr. Maroney arranged to meet in New York City with Vincent and James Haley, a partner and patent attorney at R&G. During this meeting, which was held on April 1, 2008, Dr. Drozdoff and Mr. Maroney informed them of Dr. Drozdoff's findings regarding the similarity of certain text in the Hannon Applications and the Fire application and Fire Patent, and provided Vincent and Mr. Haley with a comparison of the '097 application to the Fire Specifications. Vincent acknowledged that in filing the '097 application, he was aware that portions thereof had been copied from the published Fire Specifications. At no time during this meeting did Vincent indicate that he had ever informed the PTO, CSHL or any of the co-inventors of this fact.

59. R&G refused to assure CSHL that it would unconditionally assist CSHL in providing the PTO with all relevant facts, instead imposing on CSHL the precondition that required CSHL to first sign a waiver essentially releasing R&G from any liability for misconduct. So that CSHL could carry out its first priority to provide the PTO with full disclosure, CSHL engaged Wilmer Cutler Pickering Hale and Dorr, LLP as new counsel.

60. As a result of their malpractice, R&G and Vincent failed to obtain allowance of any of the Hannon Applications and have caused CSHL to incur substantial, unnecessary and avoidable costs for their prosecution. Not only has CSHL been damaged in the form of hundreds of thousands of dollars in legal fees paid to R&G that would not have been necessary had Vincent and R&G met their duties of care, but CSHL, having been denied patents that it otherwise would have received but for R&G's and Vincent's negligence, has lost millions of dollars in potential licensing fees for the Hannon Applications.

Vincent's Termination by R&G and Subsequent Resignation from the Practice of Law

61. In late April 2009, R&G abruptly terminated Vincent's employment.

62. The purported basis for this termination is conduct by Vincent that was the subject of a pending State of Massachusetts disciplinary proceeding at the time of Vincent's termination, as described below.

63. On or about July 20, 2009, Vincent tendered his voluntary resignation from the practice of law, as a result of said investigation. Vincent's own affidavit in support thereof acknowledged the truth of the material facts upon which the disciplinary charges were based, including the following:

a. At some time prior to April 2002, Vincent formed a business entity known as "The IP Resource Company" to perform patent database searches;

b. Vincent did not inform his partners at R&G or his clients that he was the owner and operator of The IP Resource Company;

c. Beginning in approximately April 2002 and continuing through approximately September, 2008, Vincent prepared and submitted to R&G for payment sixty separate invoices from The IP Resource Company, each invoice relating to multiple patent matters;

d. The invoices that Vincent prepared stated, in summary form, that The IP Resource Company had performed research tasks on a total of approximately 3449 separate client matters and was entitled to payment of a total of $733,771.30 for those services. The invoices did not itemize costs, services rendered, dates on which services were rendered, or time spent.

e. Vincent approved each of the sixty invoices for payment and forwarded them to R&G's accounting department.

f. Relying on Vincent's approval, R&G paid the invoices and billed the appropriate clients for the service.

g. Vincent endorsed the checks for deposit and caused them to be deposited in an account for his personal use.

h. Vincent either never maintained or did not retain the underlying billing records for the invoices submitted by the IP Resource Company, and he cannot satisfactorily account for costs incurred and services rendered.

64. CSHL was among the clients of R&G who were victimized by Vincent's conduct described above. While serving as CSHL's primary patent prosecution counsel, R&G

Andrew Fire (collectively, "Fire"), to at least, in part, describe Dr. Hannon's inventions. About one half of the "Detailed Description of Certain Preferred Embodiments" found in the three earliest filed Hannon Applications consists of text copied from the Fire application. As described below, Vincent continued to rely upon this text to describe Dr. Hannon's inventions, and in particular, the shRNA technology that is the subject of the pending Hannon Applications. By relying extensively on the copied text, Vincent failed to fully describe and distinguish Dr. Hannon's inventions from the different technology invented by Fire.

10. During the course of prosecution, Vincent and R&G filed numerous follow-up continuation and continuation-in-part ("CIP") applications, allowing several opportunities to properly re-draft the Hannon Applications in such a way that relied on an original description of Dr. Hannon's own work to accurately describe the shRNA technology that Dr. Hannon invented. Instead, R&G and Vincent continued to rely upon the text copied from the Fire application, which falsely implied that Dr. Hannon's shRNA technology was either something that Fire invented or was suggested by the Fire application.

11. R&G and Vincent compounded the prejudice resulting from their continued use of the copied Fire text by failing to properly supplement the foregoing CIP applications with Dr. Hannon's ongoing work in a timely fashion. Furthermore, neither R&G nor Vincent ever brought the fact of this copying of the Fire text, and the potential prejudice resulting from that copying, to the attention of Dr. Hannon and CSHL. Despite being aware of how their negligent conduct had compromised the Hannon Applications, R&G and Vincent continued to prosecute the applications while hiding this fact from Dr. Hannon and CSHL. Vincent and R&G further compounded their negligence by their failure to properly attribute the copied text to Fire, effectively continuing to misrepresent that disclosure as being part of Dr.

billed and collected approximately $10,000 from CSHL in the name of work allegedly performed by IP Resource Company at the request, and/or with the approval, of R&G.

FIRST CLAIM
(Legal Malpractice)

65. CSHL repeats and realleges the allegations made in paragraphs 1 through 64 above.

66. Vincent and R&G acted as attorneys for CSHL from in or around 2001 through September 2008. During this time frame, Vincent and R&G owed CSHL a duty of care.

67. During this time frame, Vincent and R&G were the only attorneys acting on behalf of CSHL in the patent prosecution of the Hannon Applications.

68. In taking the actions, and omitting to act, as described in Paragraphs 22-60 above, Mr. Vincent's and R&G's actions were negligent and fell below the applicable professional standards for representing an entity in CSHL's position in pursuing patents such as the Hannon Applications.

69. But for Vincent's and R&G's failures to satisfy their duties of care to CSHL, it would have been able to obtain allowance of claims covering Dr. Hannon's inventions.

70. As a direct and proximate result of Vincent's and R&G's negligence, CSHL has been severely damaged. CSHL has spent hundreds of thousands of dollars on legal fees that would not have been necessary had Vincent and R&G met their duties of care. These fees include substantial additional costs that CSHL has incurred resulting from its transfer of prosecution to new counsel and in legal work that new counsel has conducted in an attempt to address the harm caused by R&G's misconduct to the Hannon Applications, as well as fees paid to R&G that would not have been necessary had Vincent and R&G satisfied their duties of care.

CSHL will establish the exact amount of this category of damages at trial, but expects it to be no less than $1,000,000.

71. In addition, CSHL has lost opportunities for licensing Dr. Hannon's technology, including at minimum, lost opportunities resulting from the loss of patent term caused directly by Vincent's and R&G's malpractice. The resulting losses include lost opportunities for commercial user licenses, allowing commercial use of shRNA technology, as well as lost royalty income on research reagent sales. The exact amount of such damages will be established at trial. However, CSHL expects that the annual amount of such licensing opportunities it has lost as a direct and proximate result of Vincent's and R&G's malpractice to be at least $22,500,000 for lost commercial user license income and $9,000,000 for lost royalty income, should CSHL ultimately obtain the patent such that only five years of patent term are lost, to $57,500,000 for lost commercial user license income and $19,000,000 for lost royalty income, should CSHL be denied the patent such that fifteen years of patent term are lost.

72. In addition, as a direct and proximate result of their negligence, Vincent's and R&G's failure to obtain issuance of claims to CSHL's shRNA technology has precluded CSHL from pursuing opportunities to further commercialize this technology through a start-up company. The exact amount of such damages will be established at trial. However, CSHL expects that the start-up income it has lost as a direct and proximate result of Vincent's and R&G's malpractice to be at least $5,000,000. In sum total, depending on the number of years of term lost, CSHL expects its total damages as a direct and proximate result of Vincent's and R&G's malpractice to be at least $37,500,000 to $82,500,000.

SECOND CLAIM
(Breach of Fiduciary Duty)

73. CSHL repeats and realleges the allegations made in paragraphs 1 through 72 above.

74. As attorneys for CSHL, R&G and Vincent owed CSHL a fiduciary duty.

75. In taking the actions, and omitting to act, as described in Paragraphs 22-60 above, Mr. Vincent's and R&G's actions were in breach of their fiduciary duties to CSHL.

76. As a direct and proximate result of R&G's and Vincent's breaches of their fiduciary duties to CSHL, as described above, CSHL has been damaged in an amount to be determined at trial.

77. Specifically, CSHL has been damaged in the form of: (i) additional attorneys' fees that it has been forced to expend as a result of R&G's and Vincent's wrongful acts and omissions, which is estimated to be no less than $500,000; (ii) lost licensing opportunities for the Hannon technology, which is estimated to be worth no less than $36,500,000 to $81,500,000; and (iii) disgorgement of all attorneys' fees paid by CSHL to R&G since 2001, which is estimated to be no less than $1,400,000.

THIRD CLAIM
(Fraud and Fraudulent Concealment)

78. CSHL repeats and realleges the allegations made in paragraphs 1 through 77 above.

79. For more than eight years, while acting as primary patent prosecution counsel for CSHL, by intentionally and repeatedly concealing Vincent's copying of Fire, Vincent and R&G committed a fraud upon CSHL.

80. Vincent and R&G failed to inform CSHL of the copying of Fire, and failed to inform the PTO of the copying of Fire, despite their professional obligation to make

such disclosure. This withholding of material information was intentionally done by Vincent and R&G for the purpose of, and had the effect of, inducing CSHL to continue using Vincent and R&G as its primary patent counsel, and to reasonably rely on their advice in the mistaken belief that they had undertaken to provide professional advice in CSHL's best interests and had done nothing to stain CSHL's reputation or credibility with the PTO, or prejudice its patent applications.

81. On this point, shortly before filing the March 9, 2007 Amendment in the '557 application, Vincent went with Dr. Hannon and Mr. Maroney to meet with the examiner, essentially over the examiner's pending rejection of claims as being anticipated by Fire. Incredibly, Vincent still never brought to their attention the copying and resulting problems that he created in distinguishing Dr. Hannon's work from Fire.

82. Had Vincent been forthright about his copying of Fire anytime during the course of the prosecution of the Hannon Applications, CSHL would never have kept Vincent and R&G as its patent counsel. By intentionally concealing what actually had transpired, Vincent and R&G intentionally induced CSHL to continue using Vincent and R&G for a majority of its patent work.

83. Vincent and R&G, as attorneys for CSHL, had a duty to disclose their copying of Fire and the prejudice to the Hannon Applications that their continued reliance on the copied text had caused, as described above, which was material information.

84. As a result of this fraudulent concealment and CSHL's reasonable reliance on the deceptive statements made by Vincent and R&G (via the filed Hannon Applications) that failed to disclose the copying of Fire, CSHL has been damaged in an amount to be determined at trial, including punitive damages.

85. Vincent committed a separate fraud upon CSHL through his tendering, through R&G, of invoices from The IP Resource Company totaling at least $9587.45, all of which were paid by CSHL, notwithstanding that, upon information and belief, Vincent could not substantiate any work performed by that company for the benefit of CSHL. The statements of work performed set forth on these invoices constituted material misrepresentations.

86. Vincent tendered these invoices to CSHL, through R&G, knowing that they were false, with the intent that CSHL would rely on the statements contained in those invoices, and the fact that they were being tendered to CSHL by R&G, in making payment thereon.

87. CSHL reasonably relied on the statements of work performed contained in the invoices of The IP Resource Company invoices forwarded for payment by R&G.

88. In making payment on these fraudulent invoices, CSHL suffered damages in an amount to be determined at trial, plus punitive damages.

JURY DEMAND

Pursuant to Rule 38 of the Federal Rules of Civil Procedure, CSHL demands a trial by jury of all of its claims.

PRAYER FOR RELIEF

Wherefore, CSHL prays for judgment against R&G and Vincent as follows:

a. Granting R&G and Vincent judgment on its Claims in a sum to be determined at trial, which is expected to be no less than $37,500,000 to $82,500,000 plus punitive damages;

b. Awarding CSHL attorneys fees and costs; and

c. Awarding CSHL such other and further relief as this Court deems just and proper.

Dated: February 16, 2010
Garden City, New York

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Exhibit A

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(19) **United States**

(12) **Patent Application Publication** (10) **Pub. No.: US 2002/0162126 A1**

Beach et al. (43) **Pub. Date: Oct. 31, 2002**

(54) **METHODS AND COMPOSITIONS FOR RNA INTERFERENCE**

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(21) Appl. No.: **09/866,557**

(22) Filed: **May 24, 2001**

Related U.S. Application Data

(63) Continuation-in-part of application No. PCT/US01/08435, filed on Mar. 16, 2001.

(60) Provisional application No. 60/189,739, filed on Mar. 16, 2000. Provisional application No. 60/243,097, filed on Oct. 24, 2000.

Publication Classification

(51) **Int. Cl.**[7] **A01K 67/00**; A61K 48/00; C12N 15/87

(52) **U.S. Cl.** **800/8**; 514/44; 435/455

(57) **ABSTRACT**

The present invention provides methods for attenuating gene expression in a cell using gene-targeted double stranded RNA (dsRNA). The dsRNA contains a nucleotide sequence that hybridizes under physiologic conditions of the cell to the nucleotide sequence of at least a portion of the gene to be inhibited (the "target" gene).

Figure 1

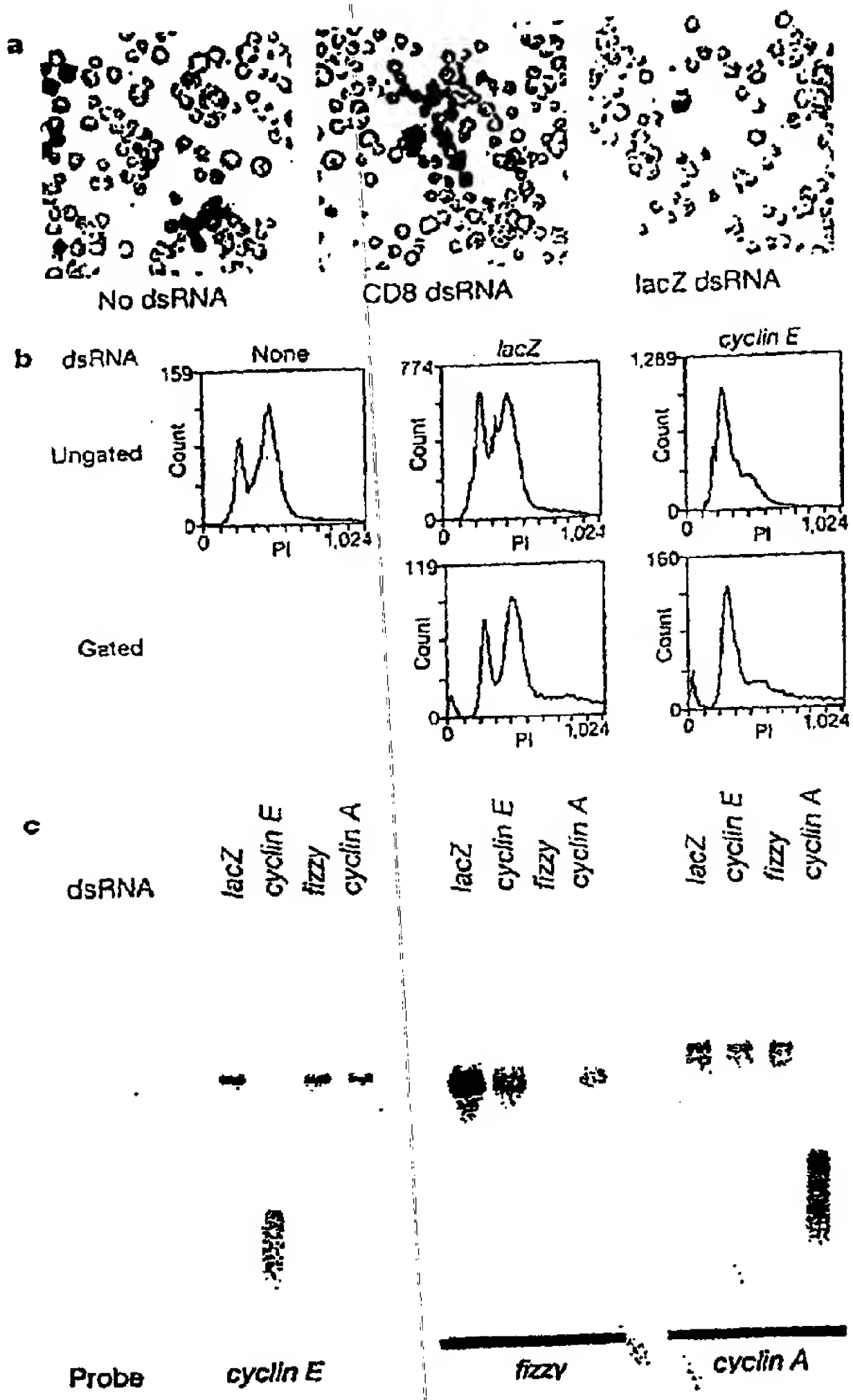
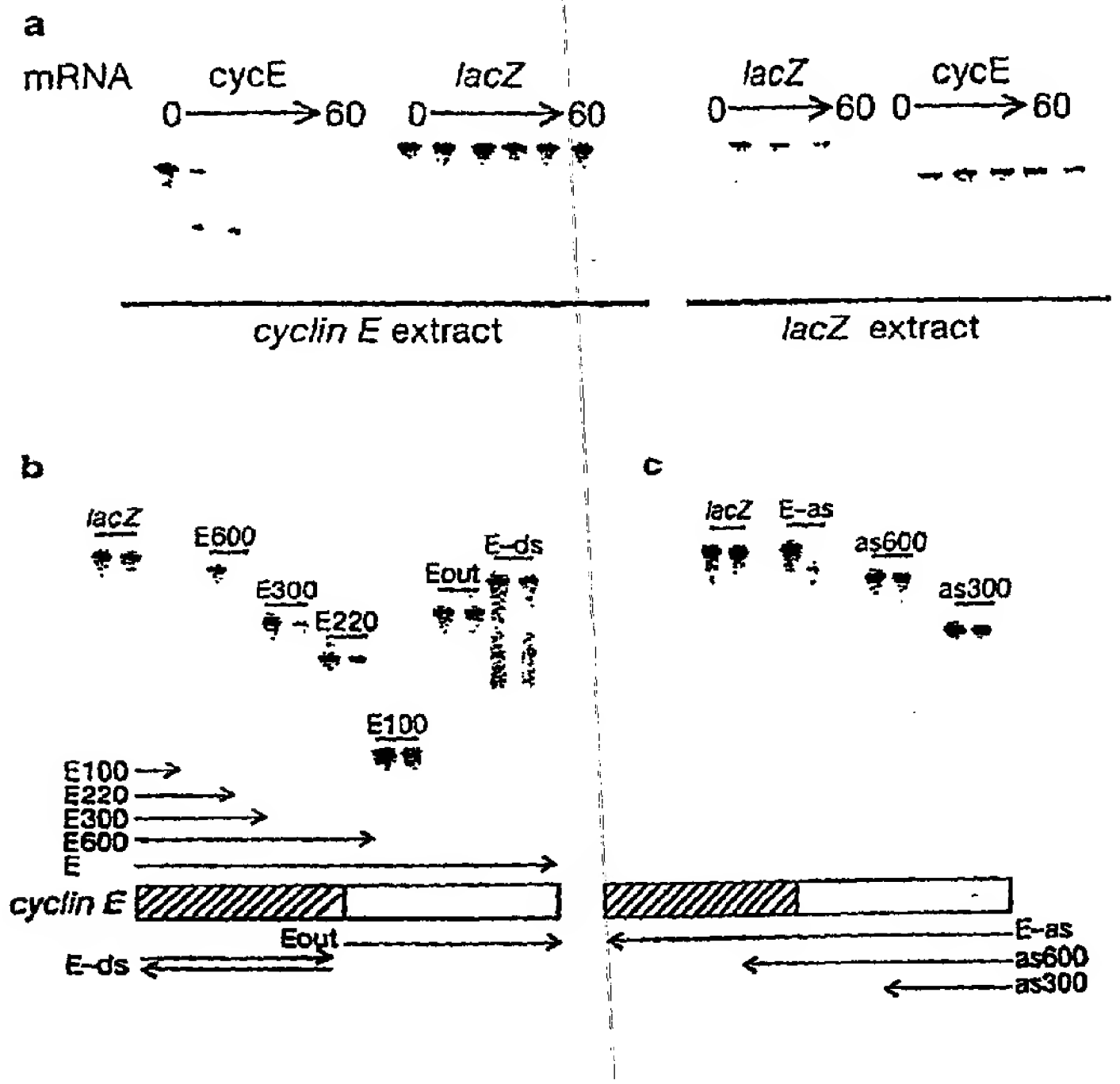

a
No dsRNA
CD8 dsRNA
lacZ dsRNA
b
dsRNA
None
lacZ
cyclin E
Ungated
Gated
Count
PI
0
1,024
159
774
1,289
119
160
c
dsRNA
lacZ
cyclin E
fizzy
cyclin A
Probe
cyclin E
fizzy
cyclin A

Figure 2


a
mRNA
cycE
0
60
lacZ
0
60
lacZ
0
60
cycE
0
60
cyclin E extract
lacZ extract
b
lacZ
E600
E300
E220
Eout
E-ds
E100
E100
E220
E300
E600
E
cyclin E
Eout
E-ds
c
lacZ
E-as
as600
as300
E-as
as600
as300

Hannon's work, and by years of delay in disclosing the Fire application to the United States Patent & Trademark Office (the "PTO"). Significantly, all this deprived Dr. Hannon and CSHL of any opportunity to mitigate the harm caused by Vincent and R&G's negligent prosecution, including the opportunity to appropriately re-draft the specification in a timely fashion to minimize any potential loss of priority.

12. It was the PTO that first noted the similarity of certain text in the Hannon Applications to that in the Fire application, a fact which ultimately brought R&G's and Vincent's misconduct to CSHL's attention. That fact, however, came to CSHL's attention only after Vincent and R&G had already caused irreparable harm through their negligent prosecution of the Hannon Applications. By the time the PTO eventually cited the Fire application as prior art against Dr. Hannon's invention, the Hannon Applications had been unfairly prejudiced by the erroneous perception that the technology invented by Dr. Hannon is not sufficiently unique from what the Fire application describes to warrant a patent.

STATEMENT OF FACTS

Background and Description of Relevant Technologies

13. CSHL is a private, not-for-profit research and education institution at the forefront of efforts in molecular biology and genetics to generate knowledge that will yield better diagnostics and treatments for cancer, neurological diseases and other major causes of human suffering.

14. Home to eight Nobelists, CSHL was founded in 1890 as one of the first institutions to specialize in genetics research and subsequently has played a central role in the seminal field of molecular biology. At CSHL in 1953, James D. Watson presented his first public lecture on his and Francis Crick's discovery of the double-helical structure of DNA, for

Figure 3

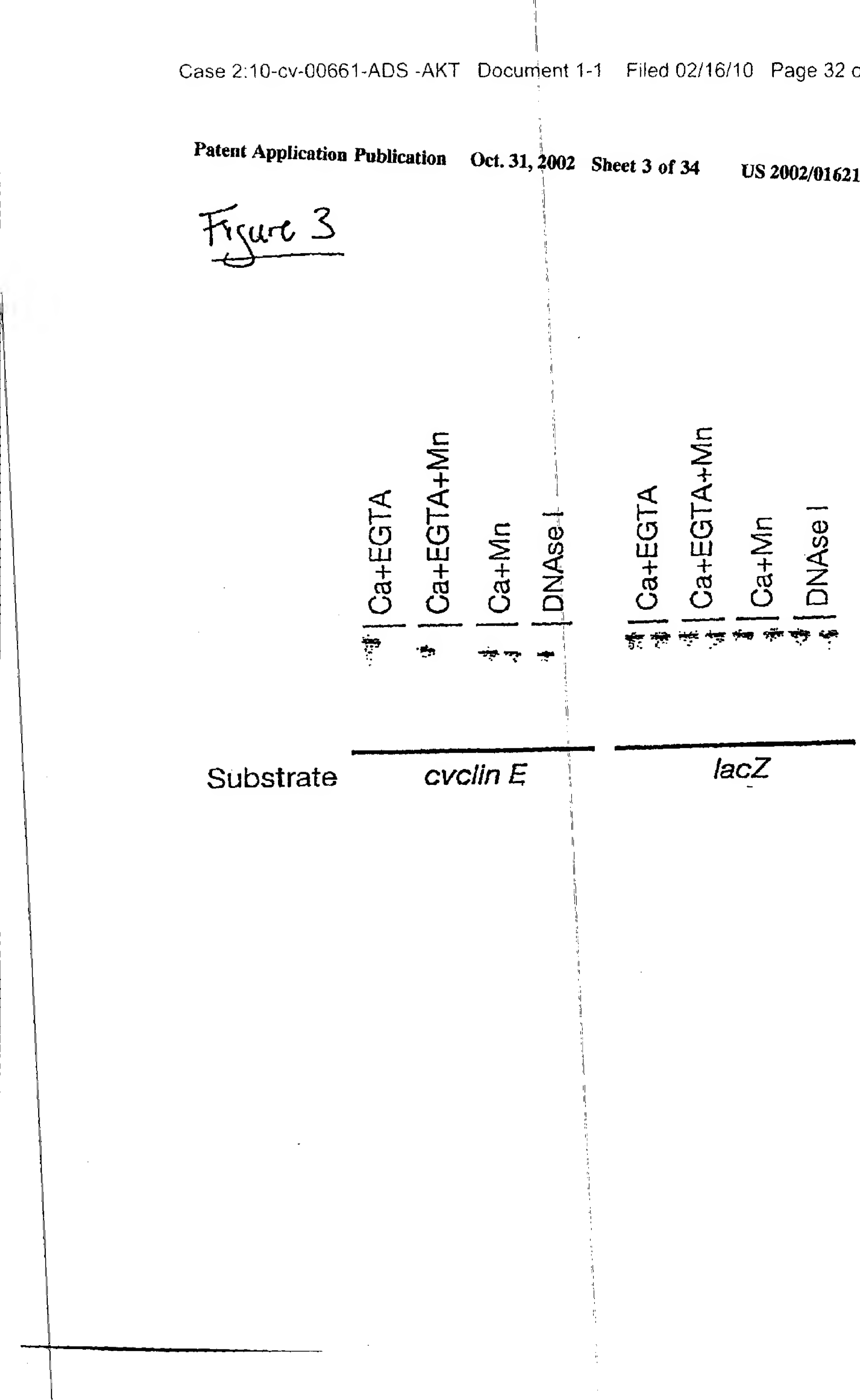
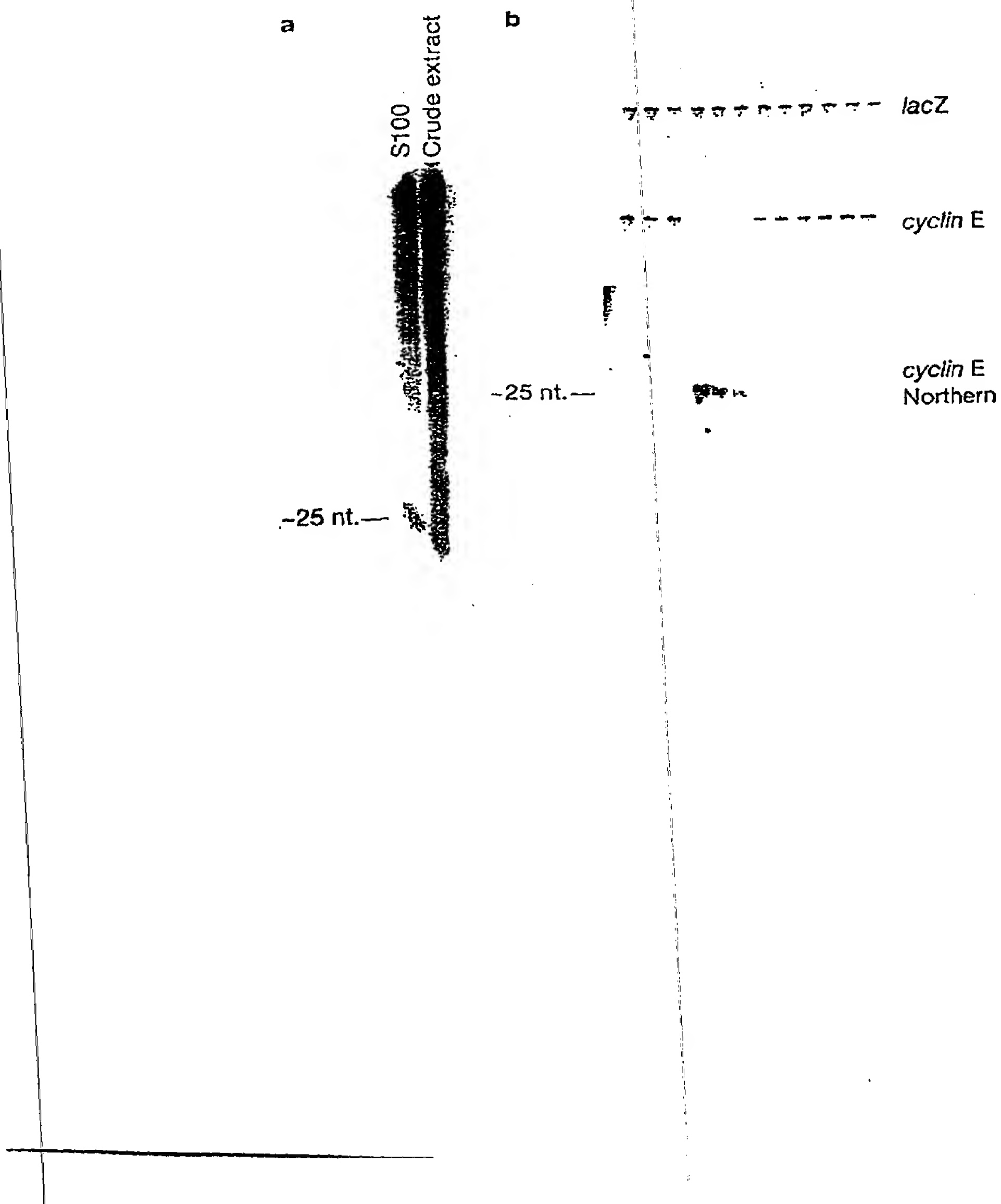

Substrate
cyclin E
lacZ
Ca+EGTA
Ca+EGTA+Mn
Ca+Mn
DNAse I
Ca+EGTA
Ca+EGTA+Mn
Ca+Mn
DNAse I

Figure 4


a
S100
Crude extract
~25 nt.
b
lacZ
cyclin E
~25 nt.
cyclin E
Northern

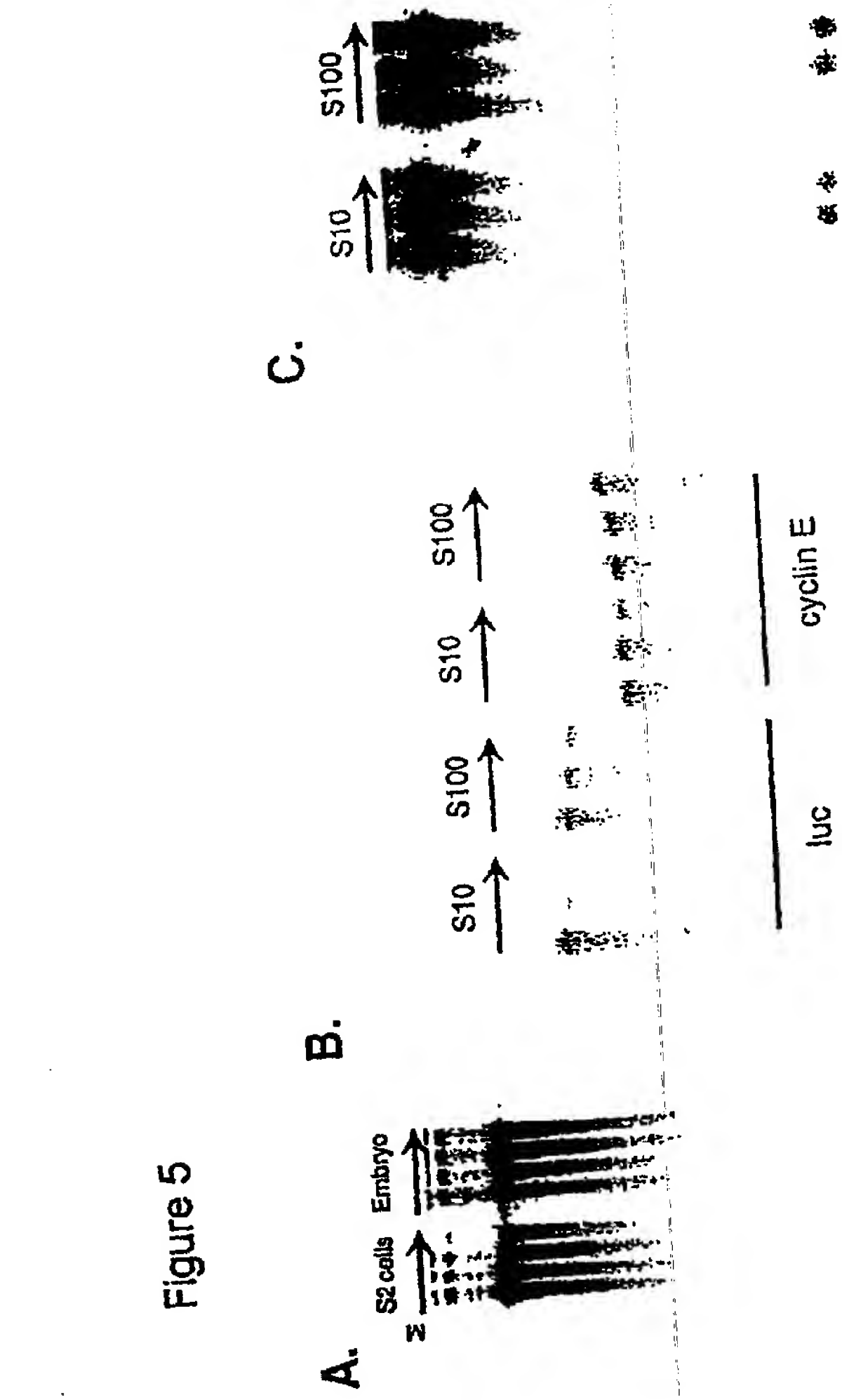


Figure 5

Figure 6a-c

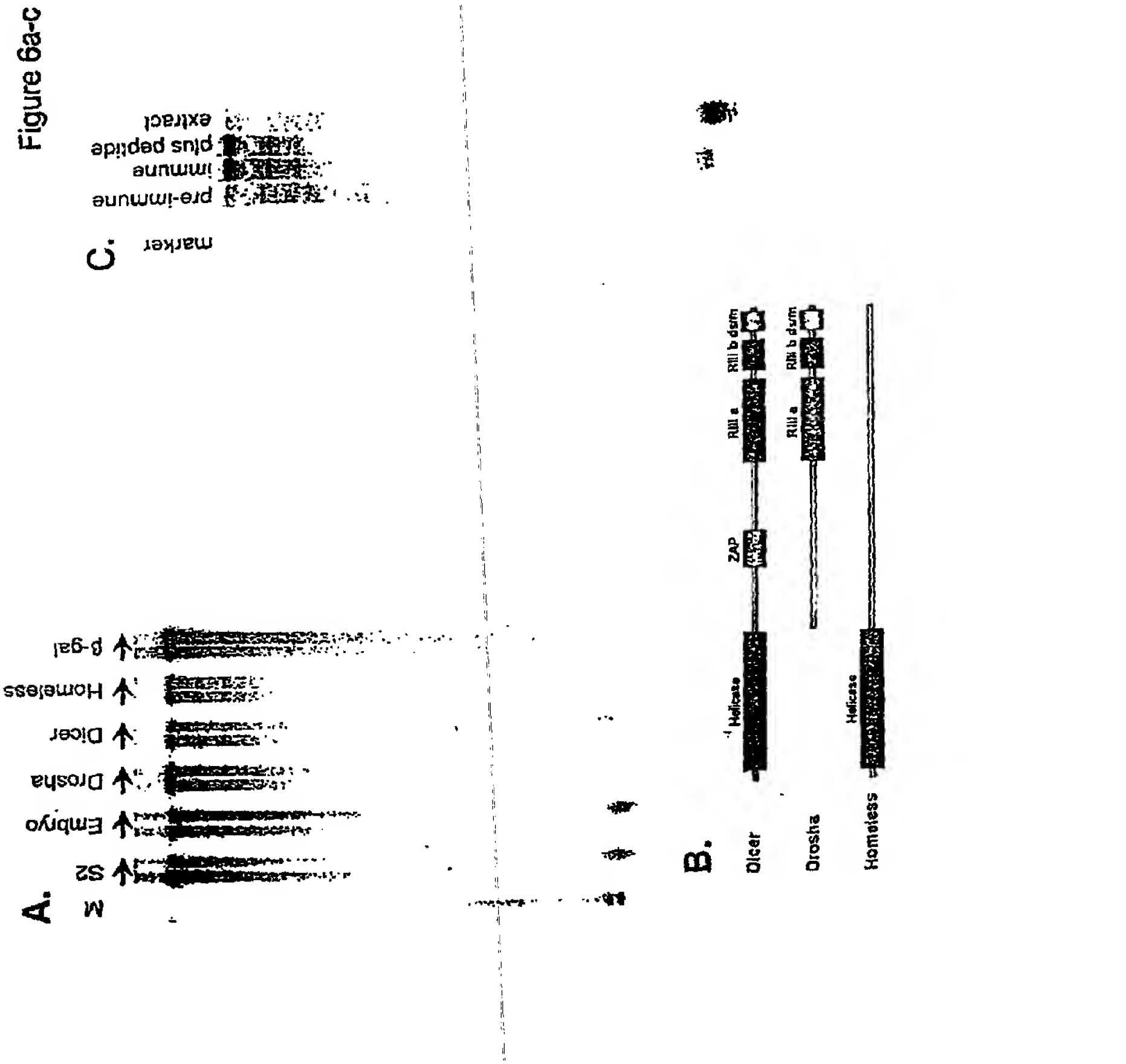

C
marker
pre-immune
immune
plus peptide
extract
A.
M
S2
Embryo
Drosha
Dicer
Homeless
β-gal
B.
Dicer
Helicase
ZAP
RIII a
RIII b dsrm
Drosha
RIII a
RIII b dsrm
Homeless
Helicase

Figure 6d-f

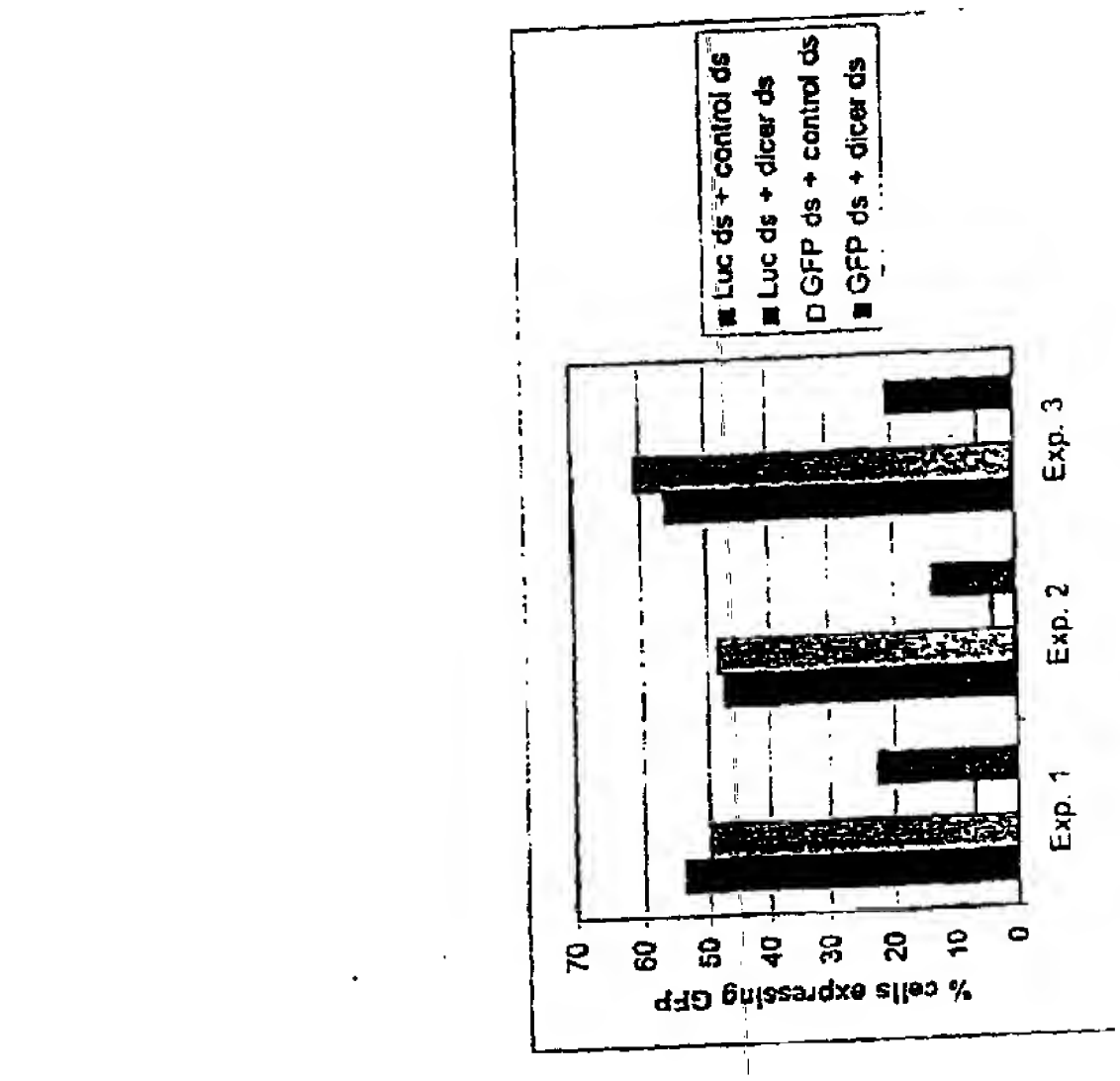
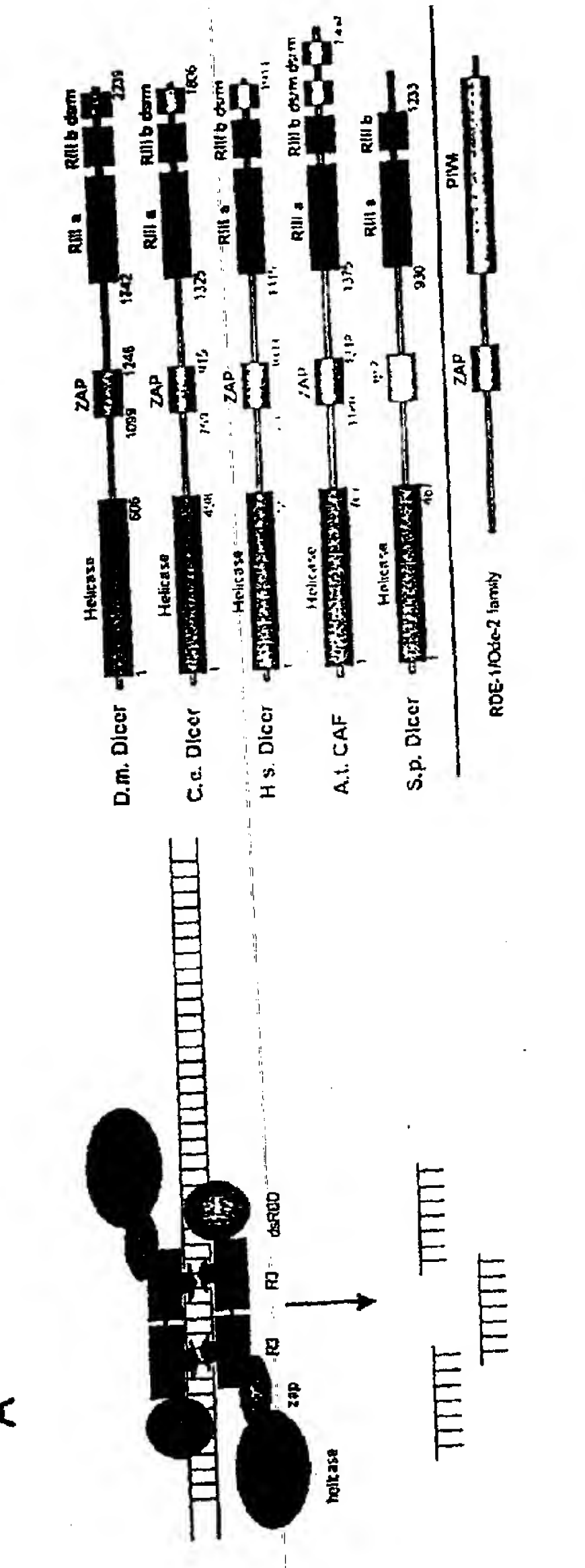

A.
casp9 dsRNA
dicer dsRNA
B.
casp9 dsRNA
dicer dsRNA
C.
% cells expressing GFP
0
10
20
30
40
50
60
70
Exp. 1
Exp. 2
Exp. 3
GFP ds + dicer ds
GFP ds + control ds
Luc ds + dicer ds
Luc ds + control ds

Figure 7

Figure 8A, B


A
dsRBD
R3
R3
zap
helicase
B
D.m. Dicer
C.e. Dicer
H s. Dicer
A.t. CAF
S.p. Dicer
Helicase
RIII a
RIII b
RIII b dsrm
dsrm
PIWI
ZAP
RDE-1/Qde-2 family

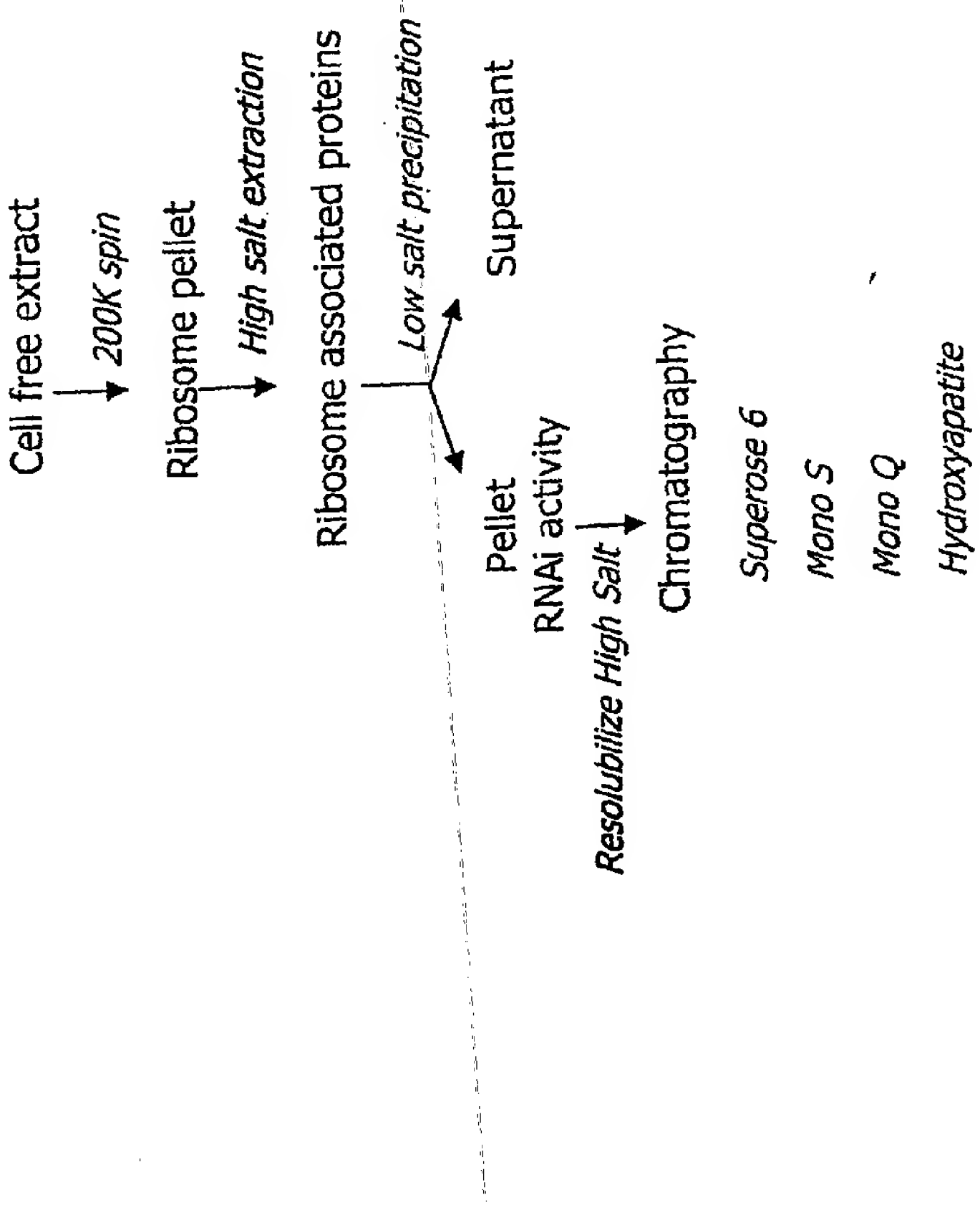

Cell free extract
200K spin
Ribosome pellet
High salt extraction
Ribosome associated proteins
Low salt precipitation
Supernatant
Pellet
RNAi activity
Resolubilize High Salt
Chromatography
Superose 6
Mono S
Mono Q
Hydroxyapatite

Figure 9

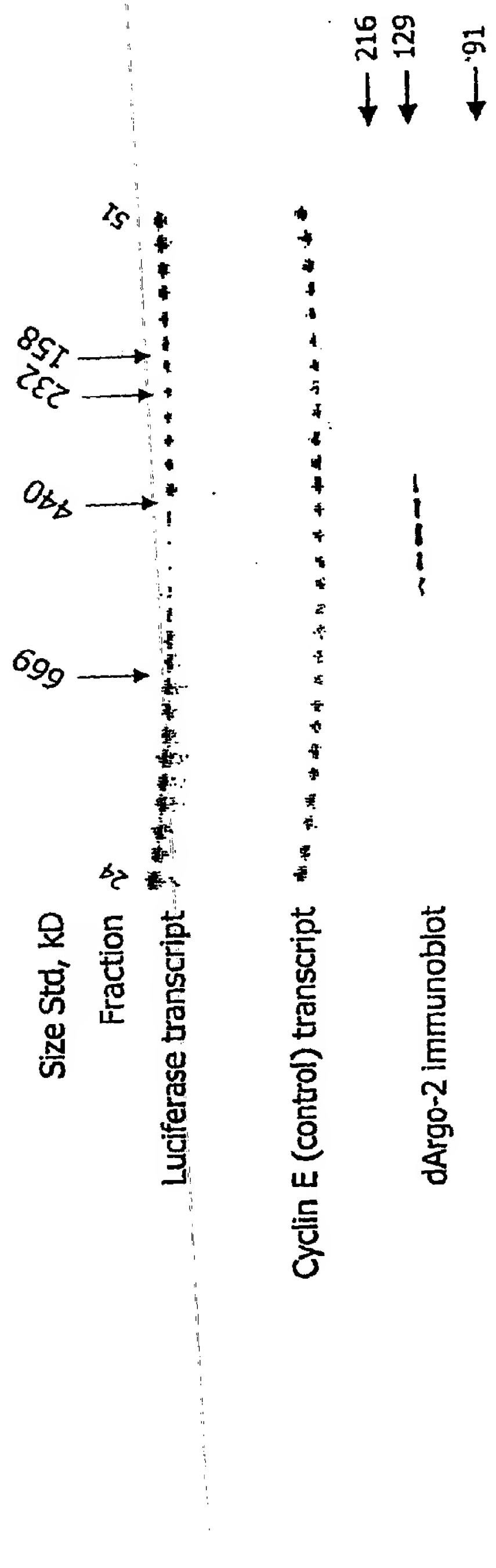

216
129
91
51
158
232
94
699
27
Size Std, kD
Fraction
Luciferase transcript
Cyclin E (control) transcript
dArgo-2 immunoblot

Figure 10

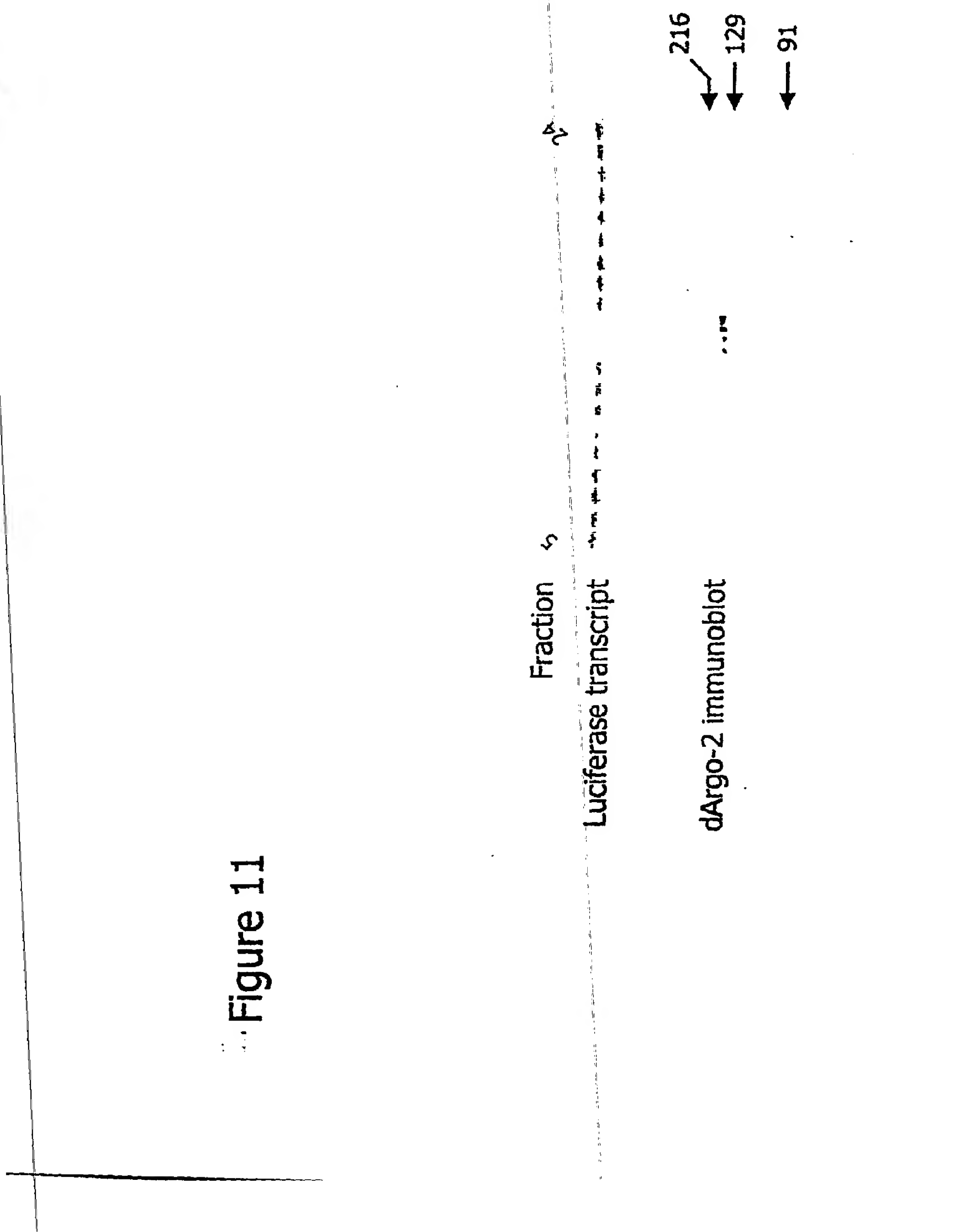

216
129
91
47
Fraction
Luciferase transcript
dArgo-2 immunoblot

Figure 11

which each later won a Nobel Prize. As Director and then President of the Laboratory from 1968 to 2003, Watson was instrumental in developing CSHL into one of the world's most influential cancer research centers.

15. Today, more than 400 scientists at CSHL pioneer the frontiers of biomedical research. A designated Center of the National Cancer Institute, CSHL has broken new ground in the study of cancer genetics. It has also taken a leading role in efforts to understand what causes neurodevelopmental and neurodegenerative illnesses such as autism, schizophrenia, and Alzheimer's and Parkinson's diseases, and is a global leader in plant genetics and in the emerging discipline of quantitative biology.

16. Each year 8,000 of the world's leading life scientists are drawn to the campus for CSHL's legendary Meetings and Courses program, where new research is discussed and debated. The CSHL Press publishes textbooks and research journals that are among the most highly cited in their fields. CSHL also has created the DNA Learning Center, the nation's first science center dedicated to public genetics education. Its hands-on programs have reached 325,000 middle and high school students, teachers, and families since 1988, and its award-winning website millions more.

17. With regard to the Hannon Applications, of particular importance are the methods and technologies Dr. Hannon invented to use shRNAs in human and other mammalian cells. Since Dr. Hannon's invention, use of shRNA for gene silencing and regulation has become a valuable and widely adopted technology, which is used today in many different fields of medical and pharmaceutical research.

18. In 2002, Dr. Hannon's research on RNA interference was recognized by Science magazine as the Breakthrough of the Year and in 2005 by Esquire as a Breakthrough of

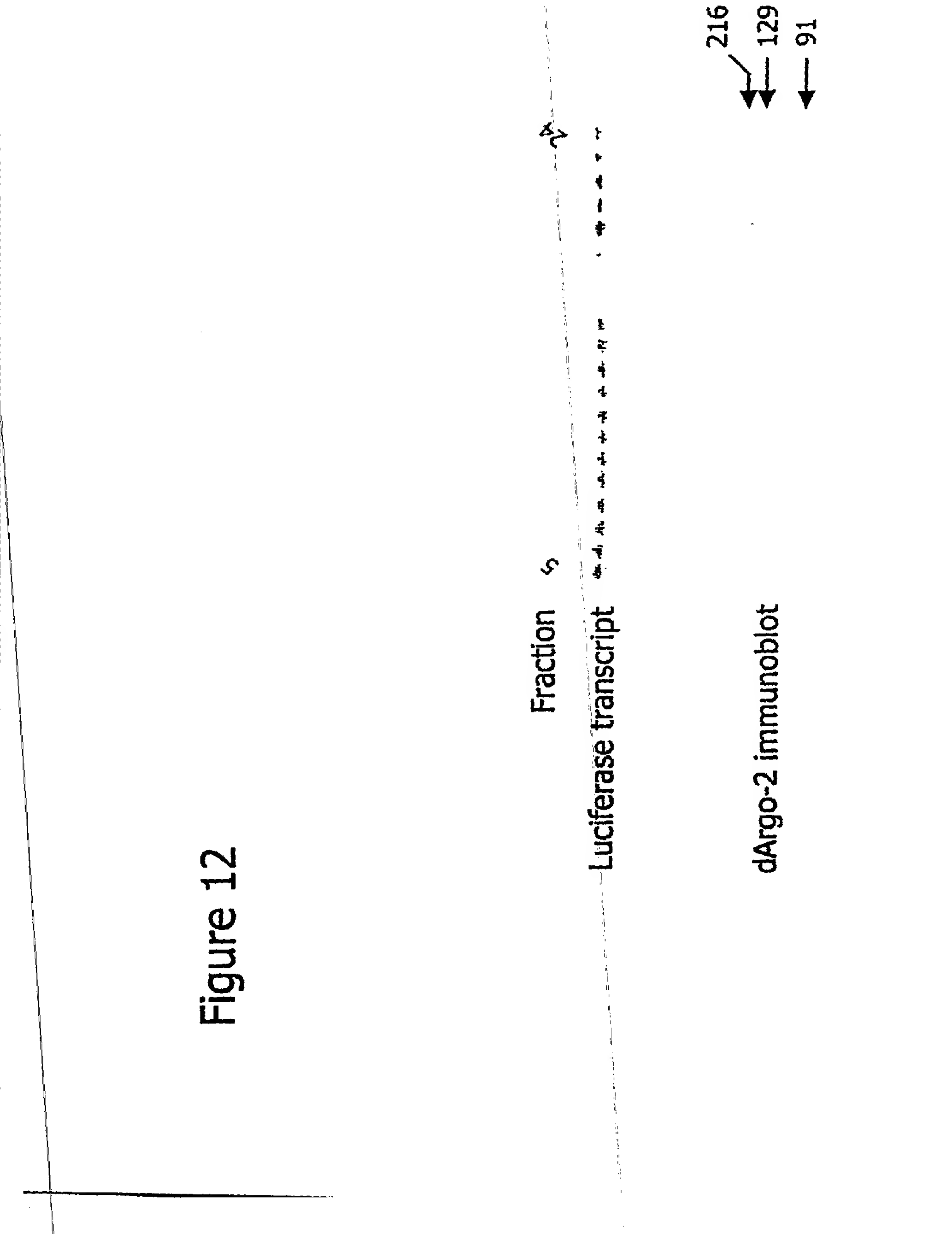

216
129
91
dArgo-2 immunoblot
Luciferase transcript
Fraction

Figure 12

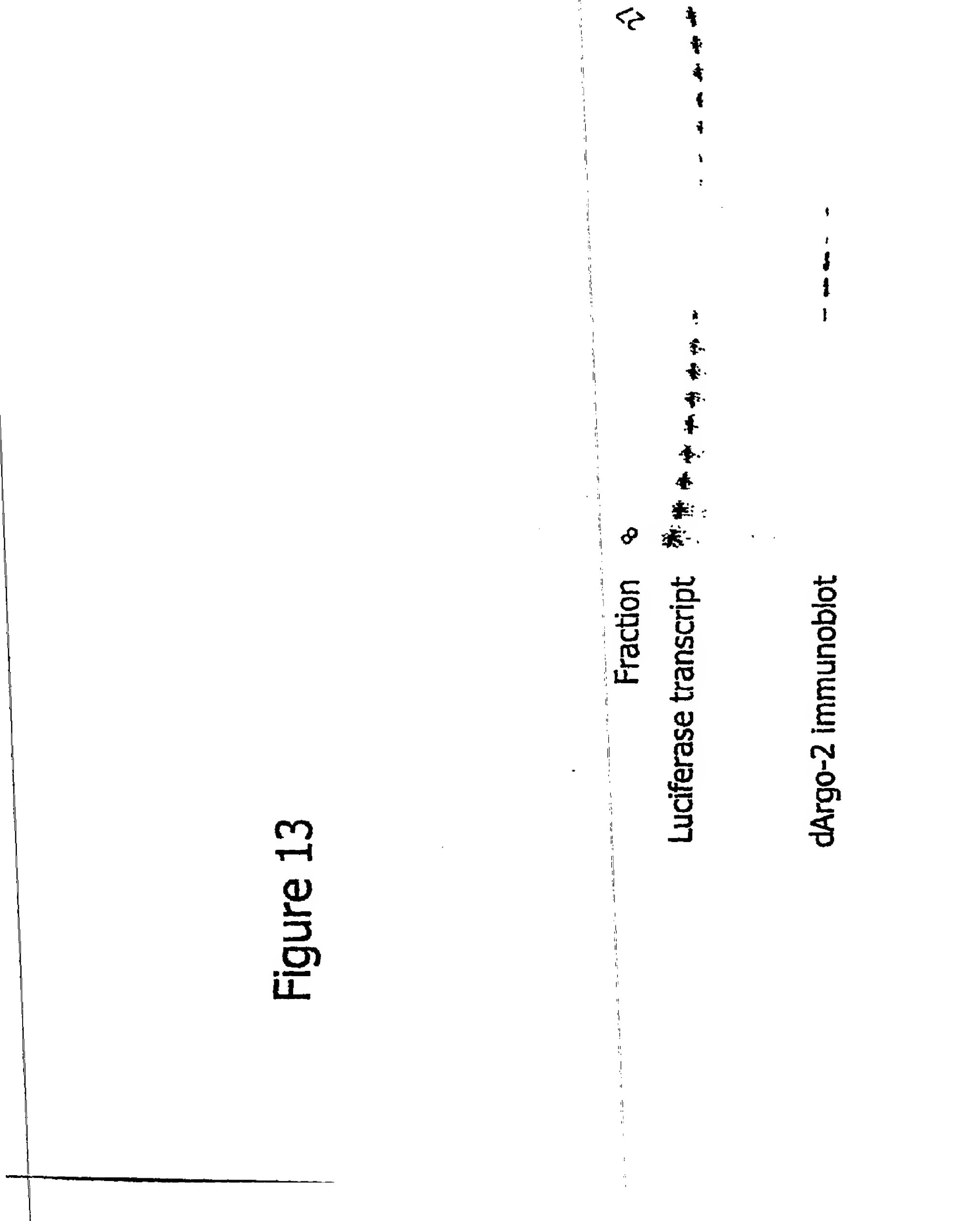


Figure 13

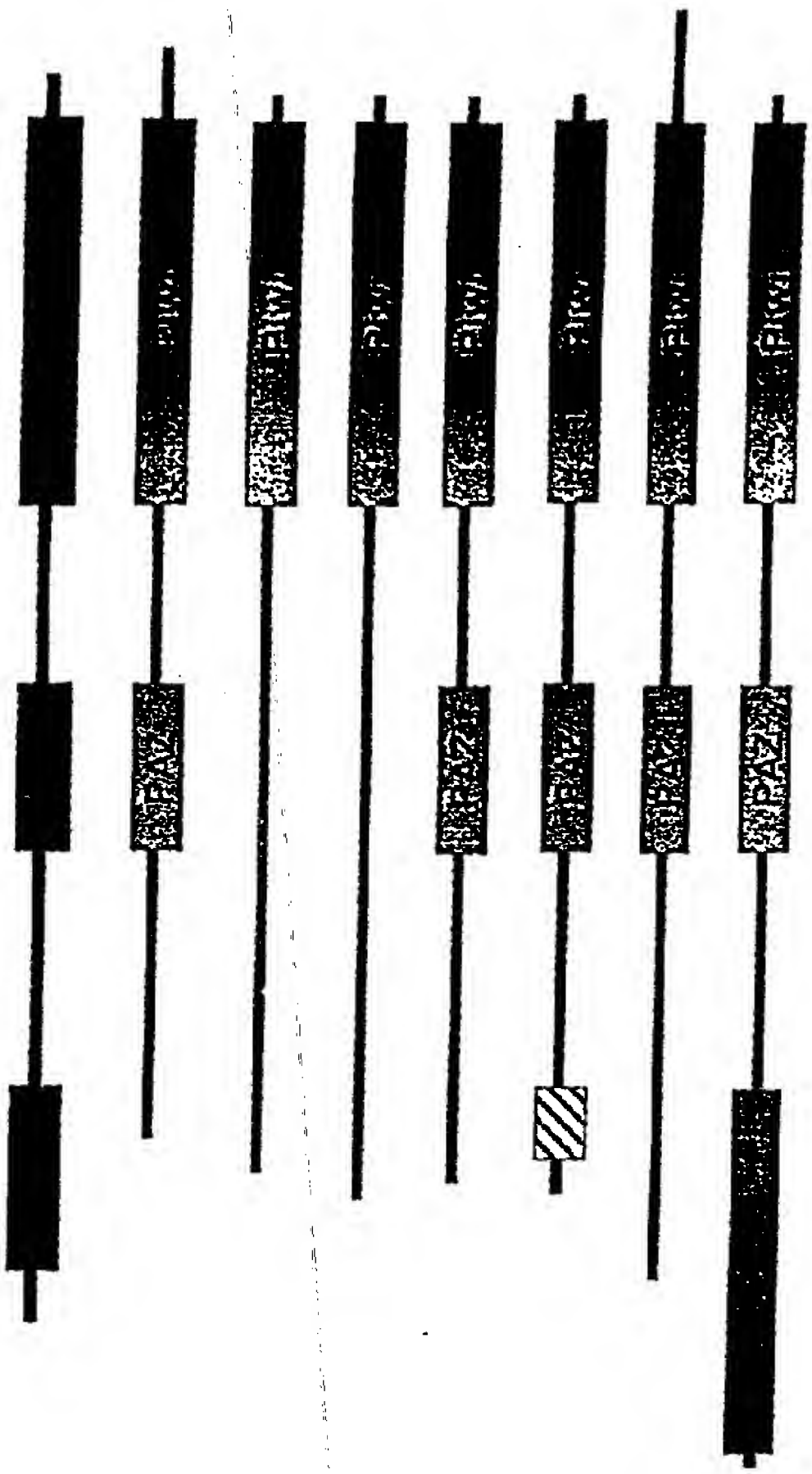

At-argonaute-1
Hs- eIF2C
Nc-qde-2
Ce-rde-1
Dm-piwi
Dm-sting
Dm-argonaute-1
Dm-argonaute-2

Figure 14

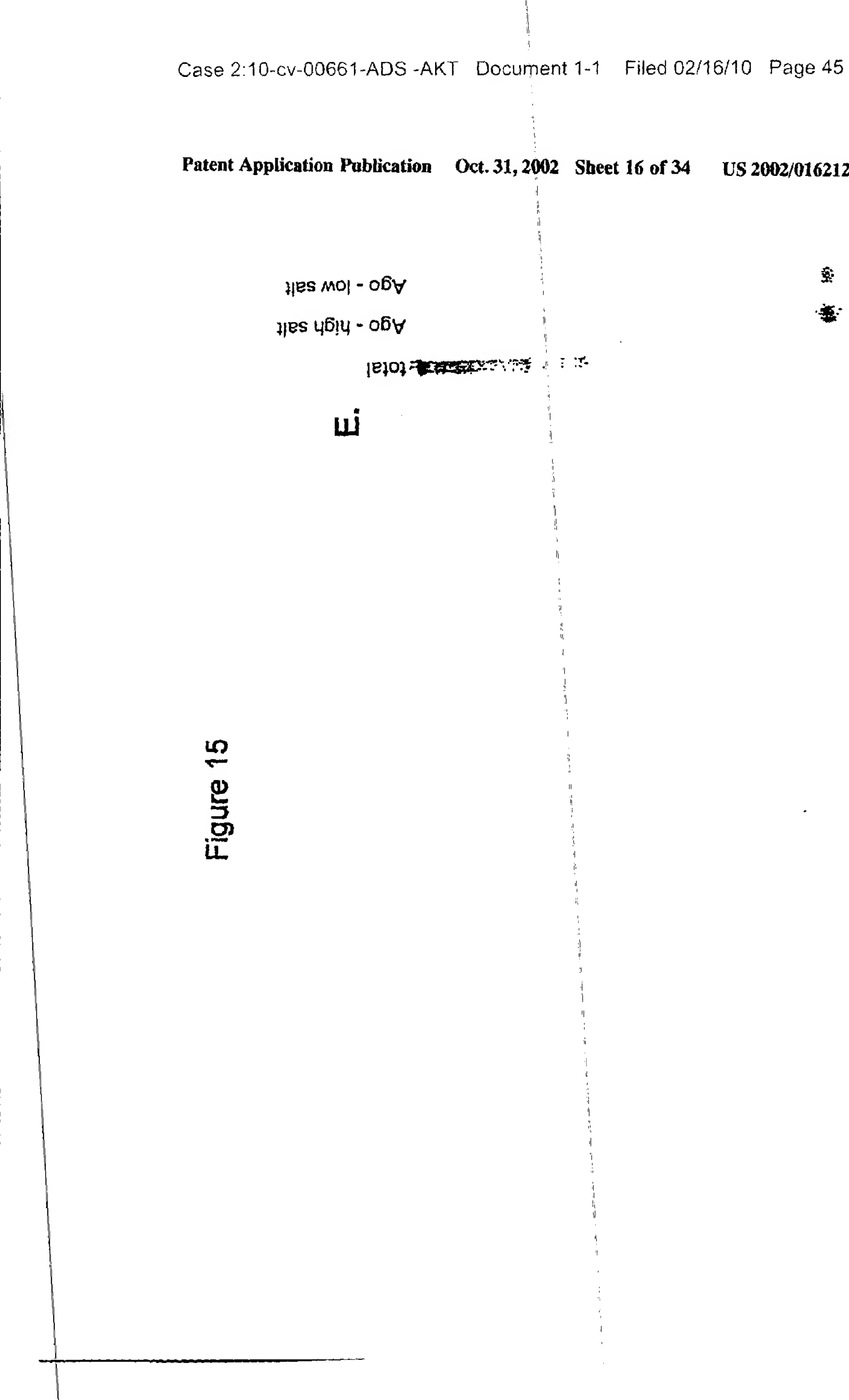
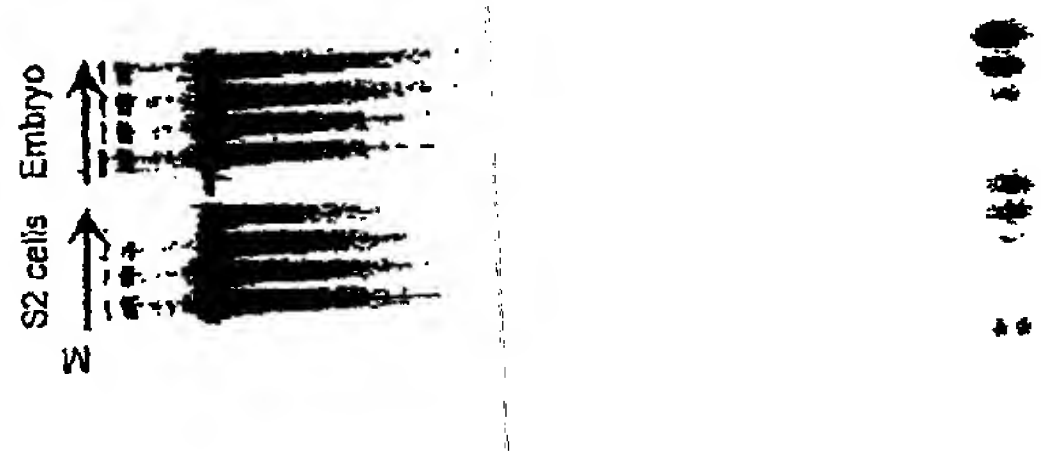
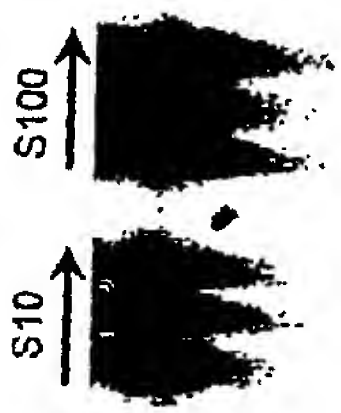


Figure 15

Figure 16





dsRNA processing

mRNA degradation

Figure 17

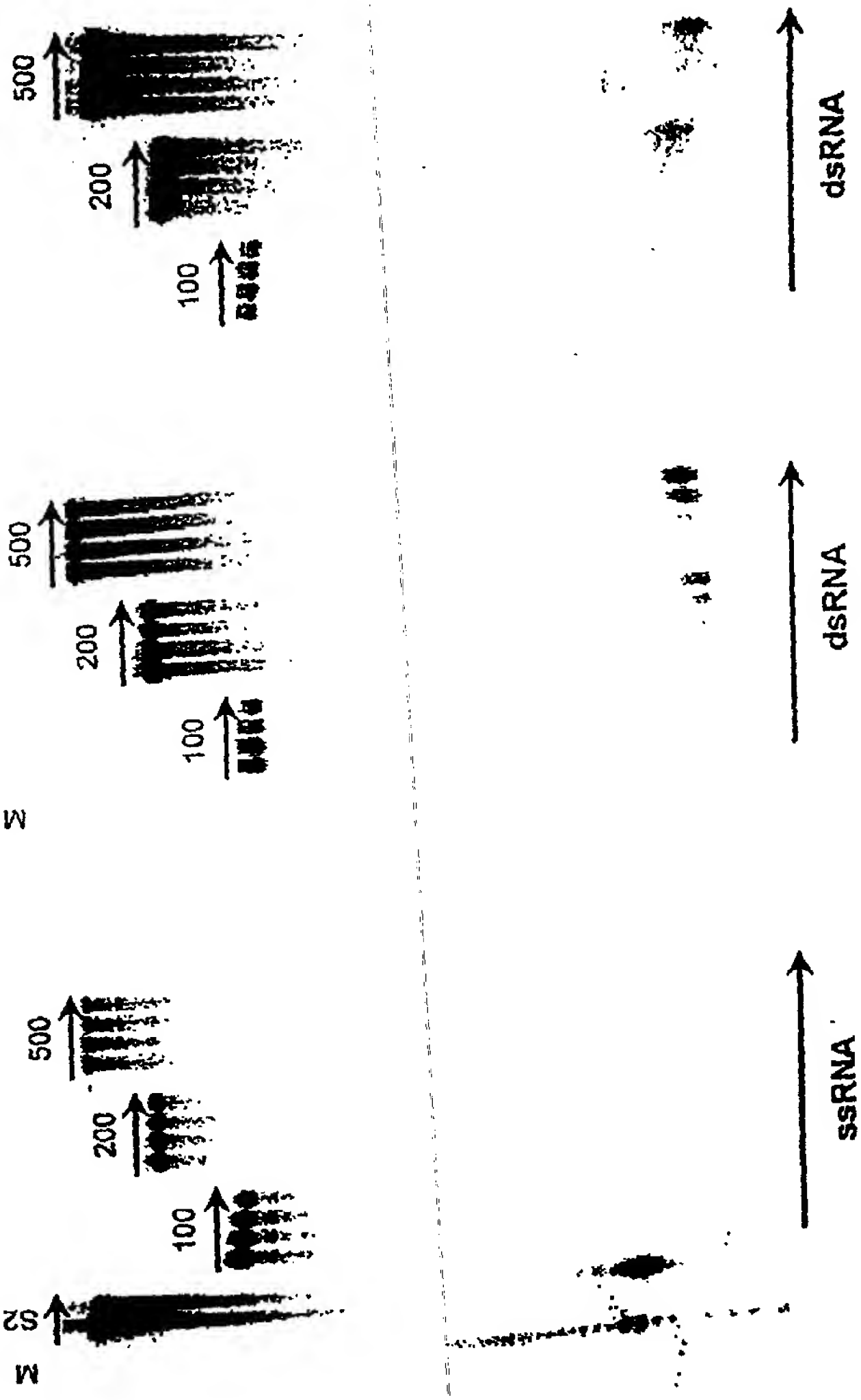


Figure 18

Figure 19

Purification of the 22-mer generating enzyme

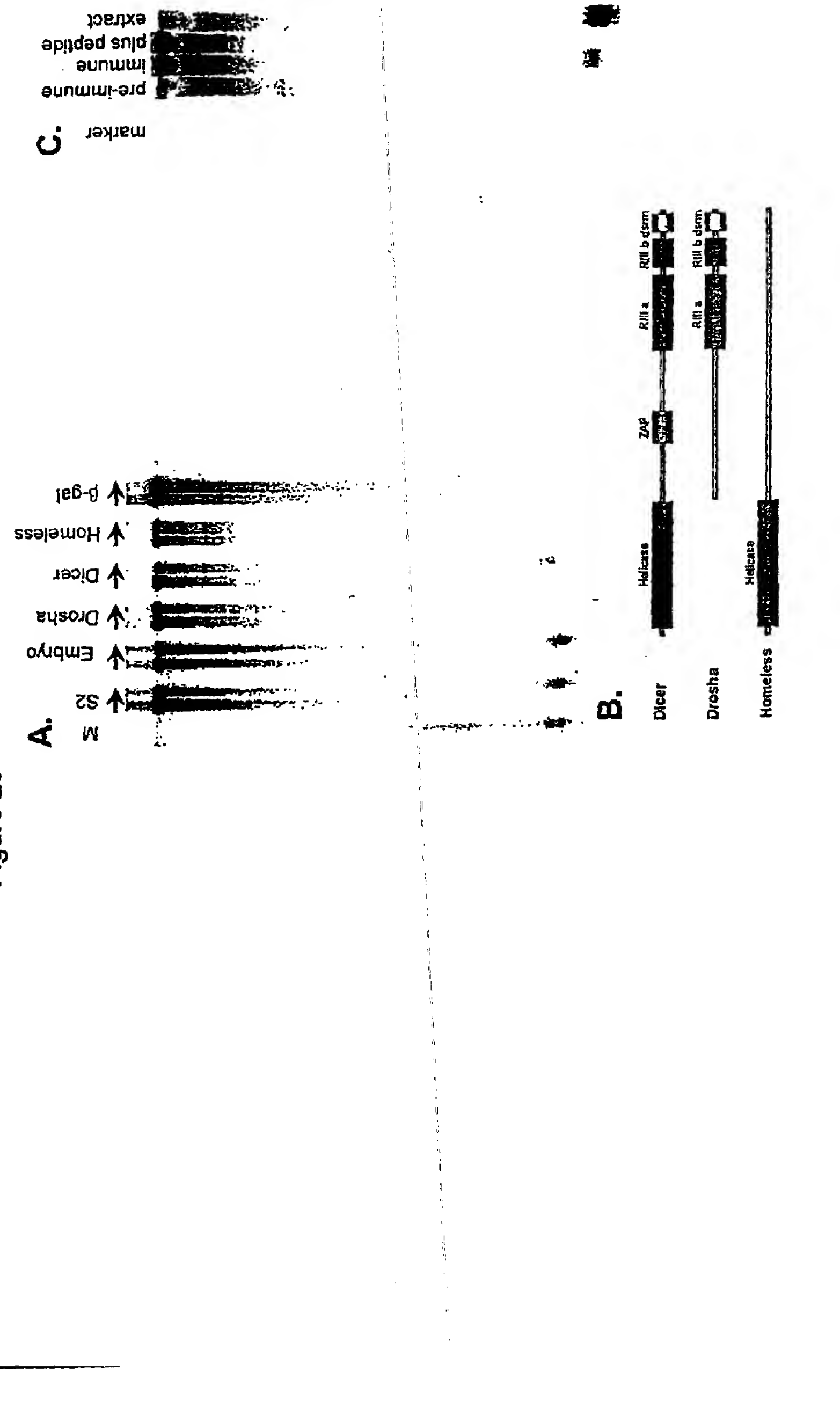
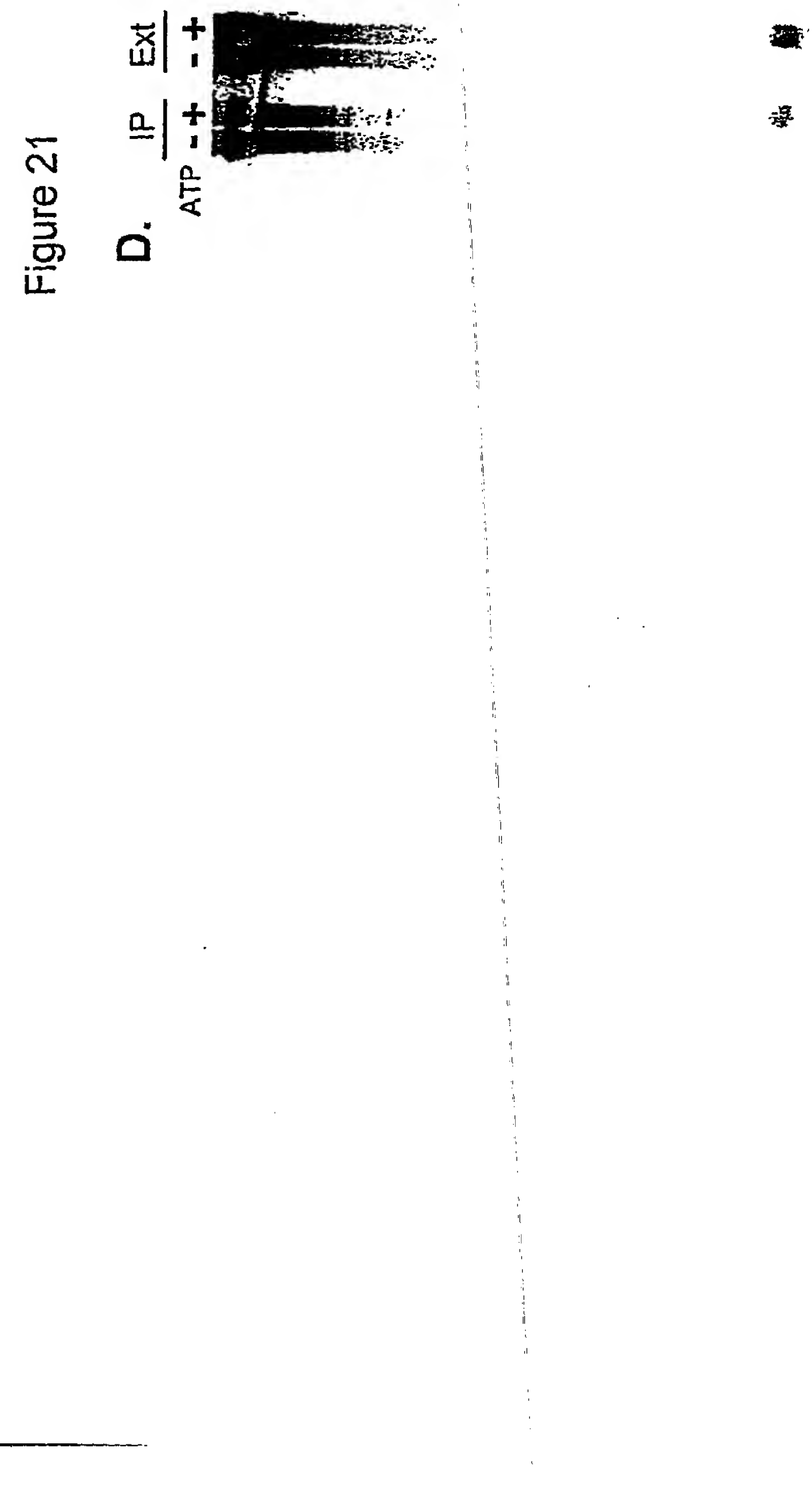

A.
M
S2
Embryo
Drosha
Dicer
Homeless
β-gal
marker
pre-immune
immune
plus peptide
extract
B.
Dicer
Drosha
Homeless
Helicase
ZAP
RIII a
RIII b dsrm

Figure 20

Figure 21



the Decade. Recognized as one of the world's most accomplished scientists, Dr. Hannon has received numerous awards, including appointment as a Pew Scholar in the Biomedical Sciences and as a Rita Allen Foundation Scholar. In 2003, he received the U.S. Army Breast Cancer Research Program's Innovator Award; in 2005 the American Association for Cancer Research's Award for Outstanding Achievement in Cancer Research and in 2007 he received the National Academy of Sciences Award for Molecular Biology and The Memorial Sloan-Kettering Cancer Center's Paul Marks Prize for Cancer Research. He assumed his current position in 2005 as a Howard Hughes Medical Institute Professor and continues to explore the mechanisms and regulation of RNA interference as well as its applications to cancer research.

19. CSHL is the assignee of the entire right, title, and interest in the Hannon Applications, which collectively refer to U.S. patent application numbers 09/858,862 filed May 16, 2001 ("the '862 application"), 09/866,557, filed March 24, 2001 ("the '557 application), 10/055,797 filed January 22, 2002 ("the '797 application"), 10/350,798 filed January 24, 2003, 10/997,086, filed November 23, 2004, 11/791,554 filed May 23, 2007, 11/894,676 filed August 20, 2007, 12/152,655 filed May 15, 2008, 12/152,837 filed May 16, 2008 and international patent applications PCT/US01/08435 filed March 16, 2001 ("the '435 PCT application"), PCT/US03/01963 filed January 22, 2003, and PCT/US05/42488 filed November 23, 2005, including all foreign patent applications filed therefrom. Certain of the Hannon Applications claim a benefit of priority to U.S. provisional applications 60/189,739 filed March 16, 2000 ("the '739 application"), and 60/243,097 filed October 24, 2000 ("the '097 application).

20. International Patent Application PCT/US98/27233, which was published on July 1, 1999 with International Publication Number WO/99/32619 (the "Fire application"), describes certain work conducted by Fire relating to his discovery that long double stranded

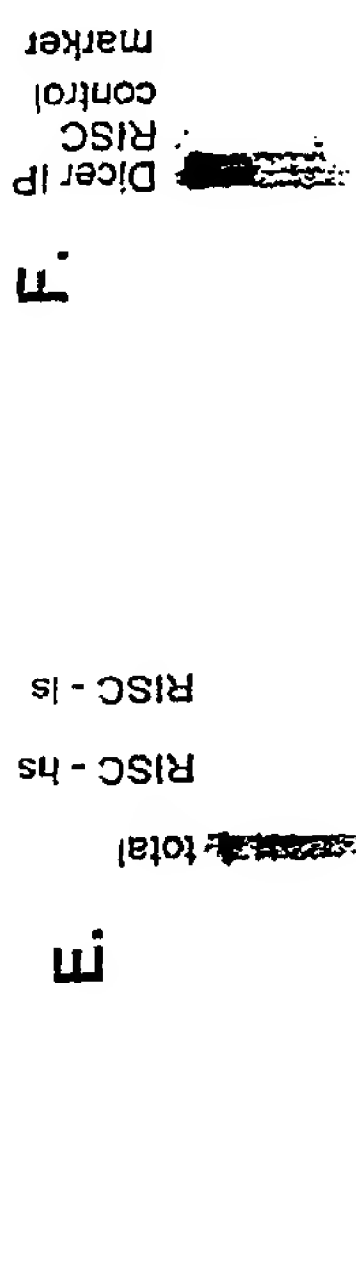


Figure 22

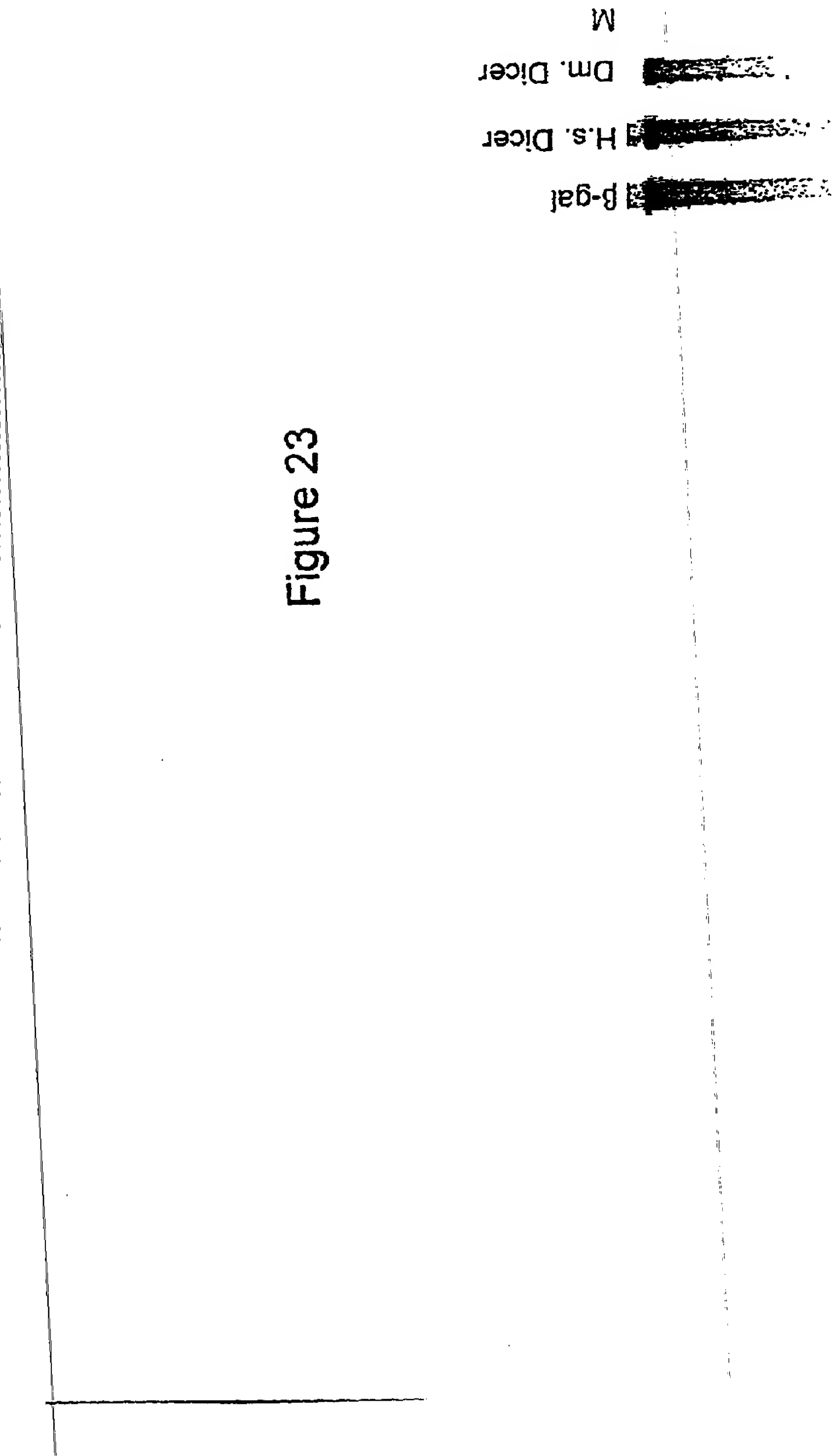


Figure 23

Figure 24

MGKKDKNKKGGQDSAAAPQPQQQQKQQQQRQQQPQQLQQPQQLQQPQQLQQPQQQQQQ
QPHQQQQQSSRQQPSTSSGGSRASGFQQGGQQQKSQDAEGWTAQKKQGKQQVQGWTKQ
GQQGGHQQGRQGQDGGYQQRPPGQQQGGHQQGRQGQEGGYQQRPPGQQQGGHQQGRQG
QEGGYQQRPSGQQQGGHQQGRQGQEGGYQQRPPGQQQGGHQQGRQGQEGGYQQRPSGQ
QQGGHQQGRQGQEGGYQQRPSGQQQGGHQQGRQGQEGGYQQRPSGQQQGGHQQGRQGQ
EGGYQQRPPGQQPNQTQSQGQYQSRGPPQQQQAAPLPLPPQPAGSIKRGTIGKPGQVG
INYLDLDLSKMPSVAYHYDVKIMPERPKKFYRQAFEQFRVDQLGGAVLAYDGKASCYS
VDKLPLNSQNPEVTVTDRNGRTLRYTIEIKETGDSTIDLKSLTTYMNDRIFDKPMRAM
QCVEVVLASPCHNKAIRVGRSFFKMSDPNNRHELDDGYEALVGLYQAFMLGDRPFLNV
DISHKSFPISMPMIEYLERFSLKAKINNTTNLDYSRRFLEPFLRGINVVYTPPQSFQS
APRVYRVNGLSRAPASSETFEHDGKKVTIASYFHSRNYPLKFPQLHCLNVGSSIKSIL
LPIELCSIEEGQALNRKDGATQVANMIKYAATSTNVRKRKIMNLLQYFQHNLDPTISR
FGIRIANDFIVVSTRVLSPPQVEYHSKRFTMVKNGSWRMDGMKFLEPKPKAHKCAVLY
CDPRSGRKMNYTQLNDFGNLIISQGKAVNISLDSDVTYRPFTDDERSLDTIFADLKRS
QHDLAIVIIPQFRISYDTIKQKAELQHGILTQCIKQFTVERKCNNQTIGNILLKINSK
LNGINHKIKDDPRLPMMKNTMYIGADVTHPSPDQREIPSVVGVAASHDPYGASYNMQY
RLQRGALEEIEDMFSITLEHLRVYKEYRNAYPDHIIYYRDGVSDGQFPKIKNEELRCI
KQACDKVGCKPKICCVIVVKRHHTRFFPSGDVTTSNKFNNVDPGTVVDRTIVHPNEMQ
FFMVSHQAIQGTAKPTRYNVIENTGNLDIDLLQQLTYNLCHMFPRCNRSVSYPAPAYL
AHLVAARGRVYLTGTNRFLDLKKEYAKRTIVPEFMKKNPMYFV

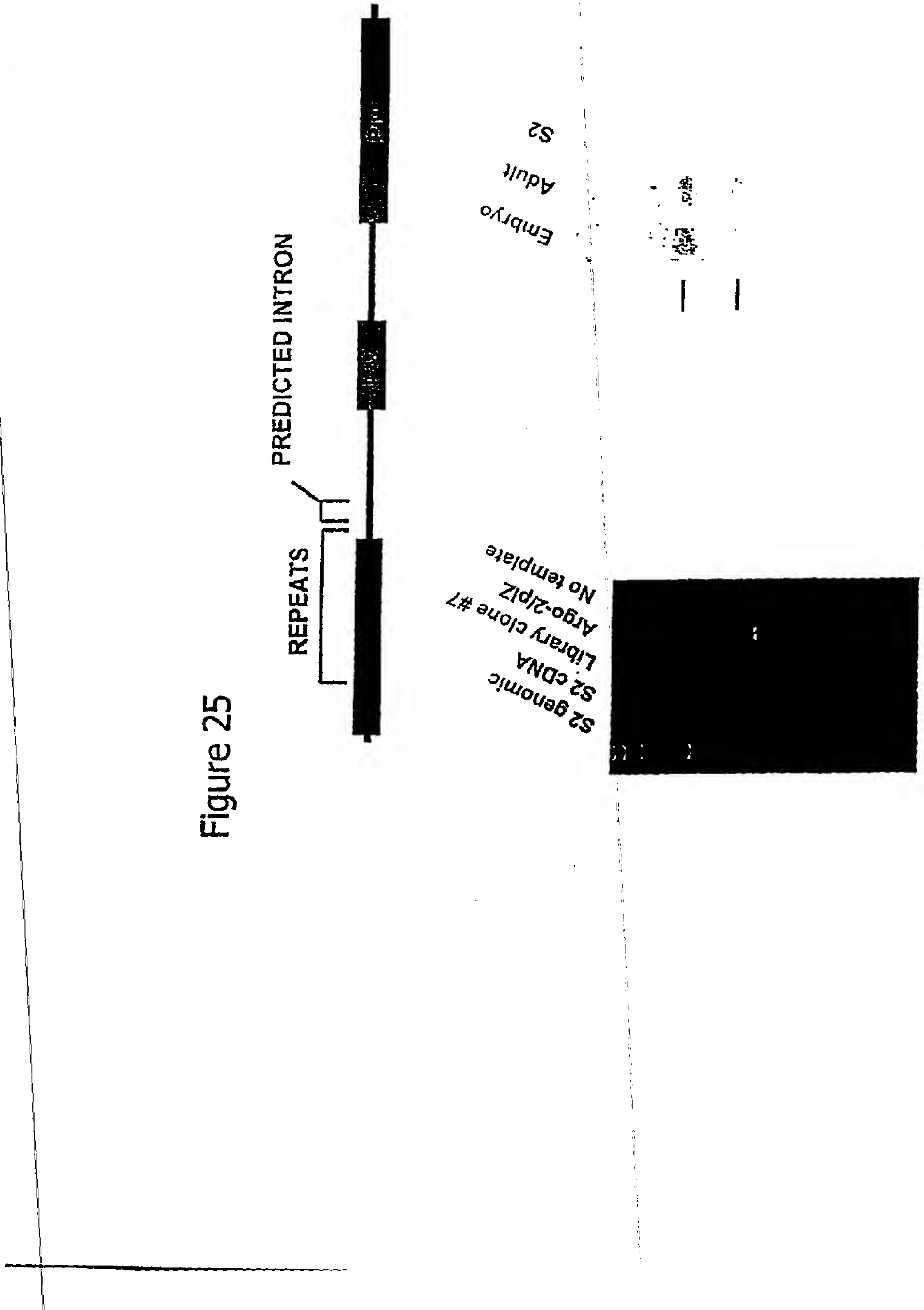

S2
Adult
Embryo
PREDICTED INTRON
REPEATS
S2 genomic
S2 cDNA
Library clone #7
Argo-2/pI2
No template

Figure 25

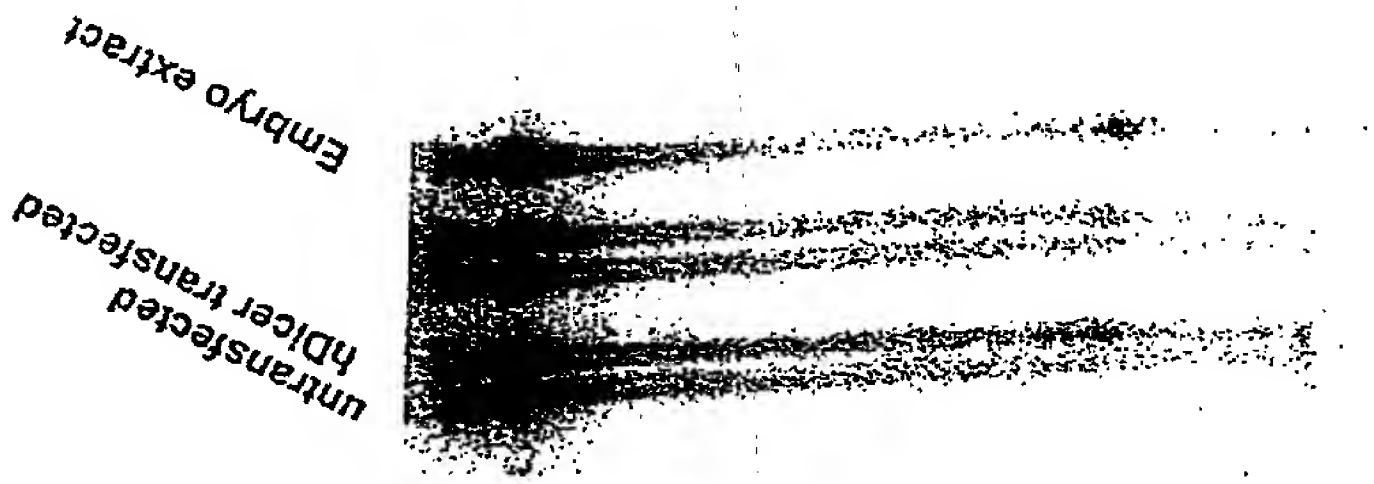


Figure 26

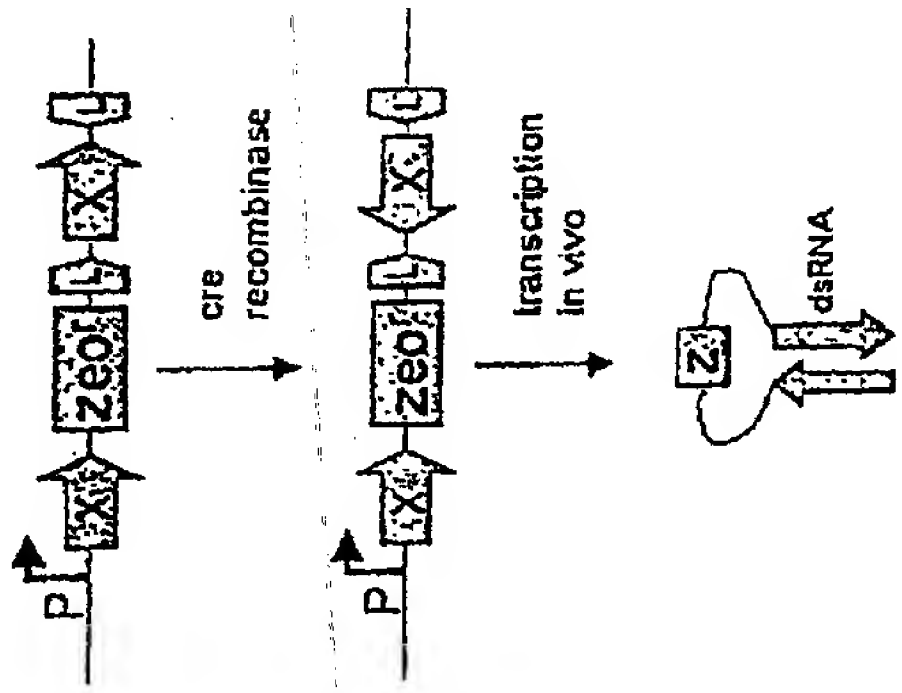


Figure 27

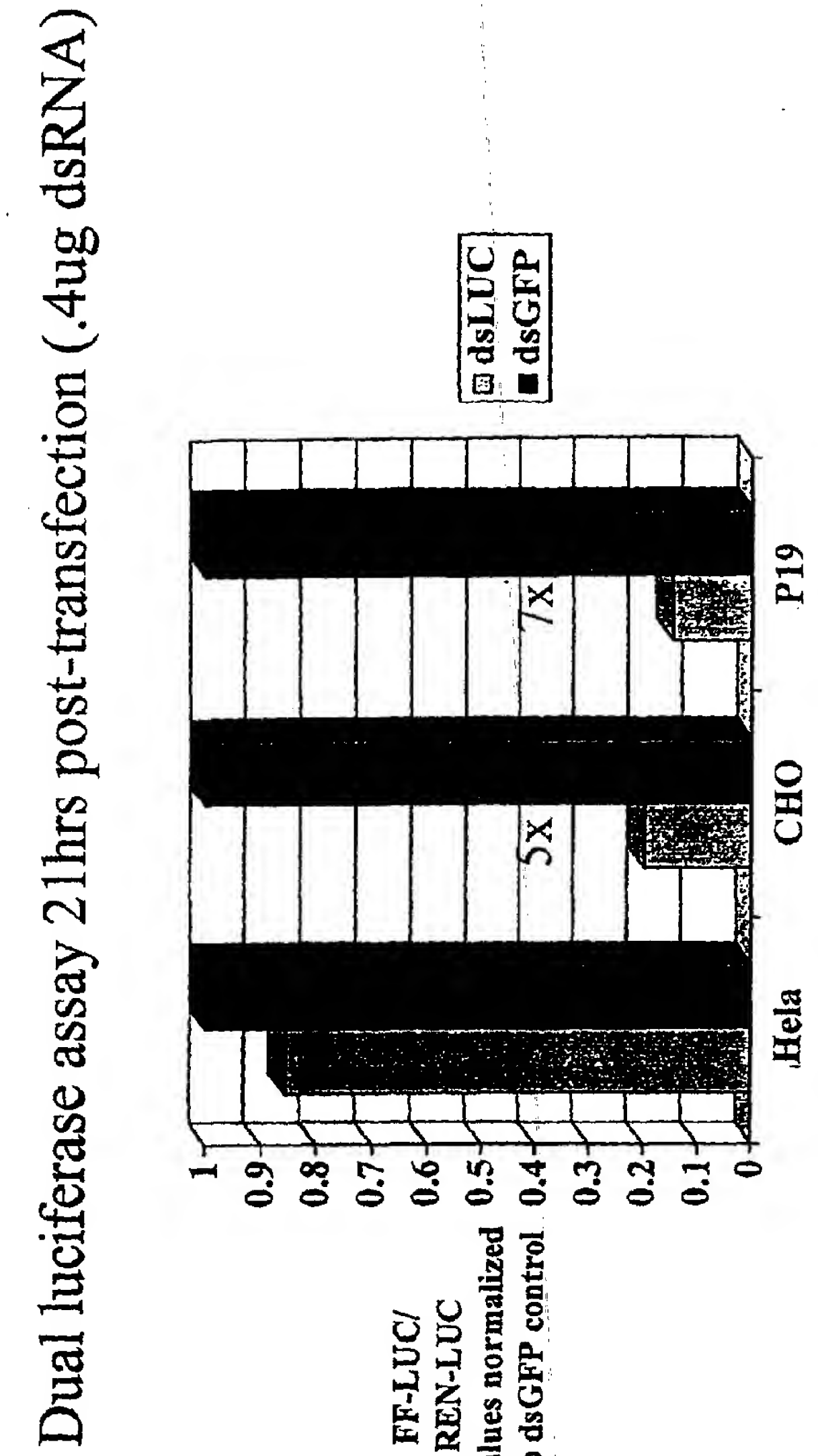

Dual luciferase assay 21hrs post-transfection (.4ug dsRNA)
dsLUC
dsGFP
FF-LUC/
REN-LUC
Values normalized
to dsGFP control
1
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
5X
1X
Hela
CHO
P19

Figure 28

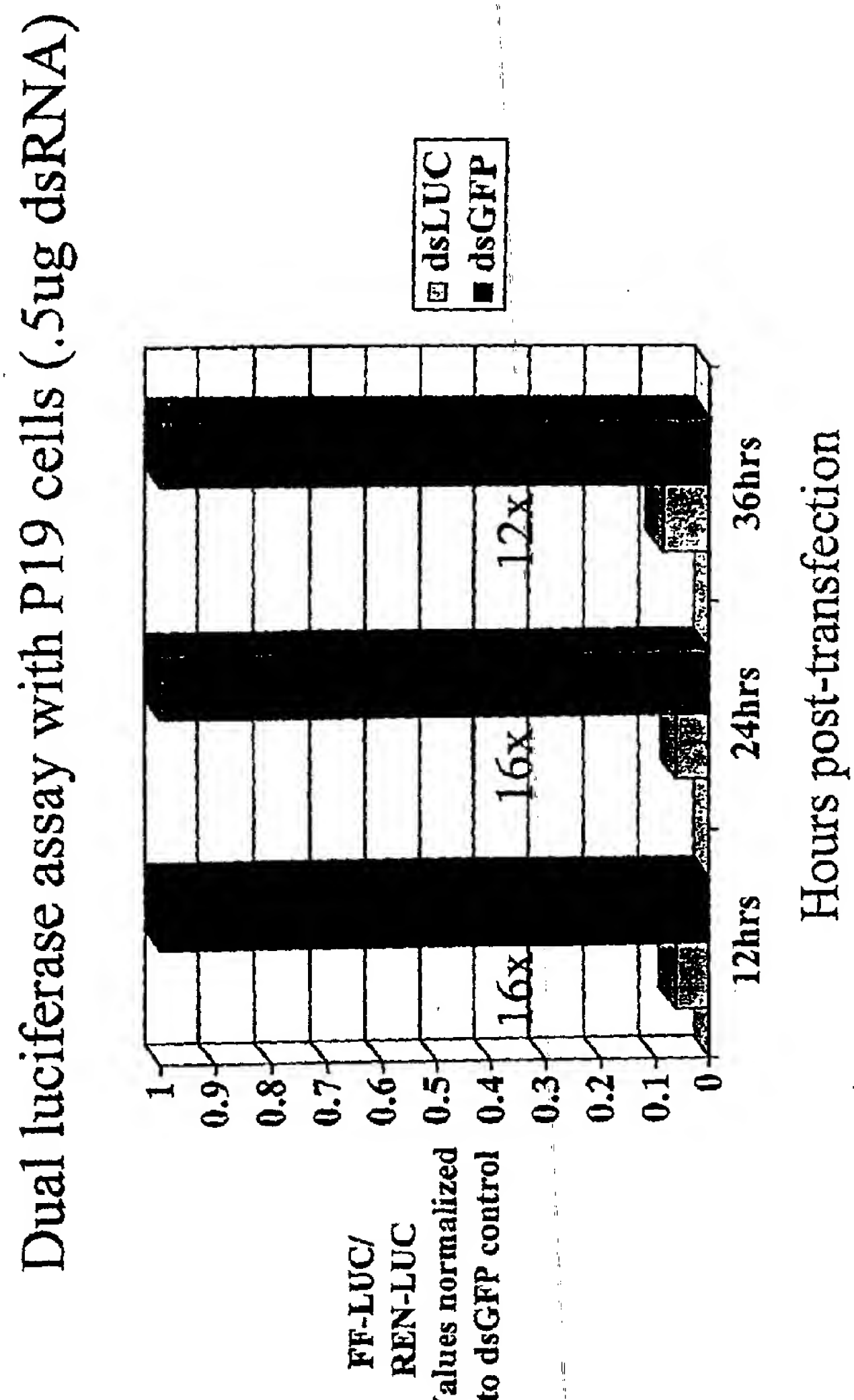

Dual luciferase assay with P19 cells (.5ug dsRNA)
dsLUC
dsGFP
FF-LUC/
REN-LUC
Values normalized
to dsGFP control
1
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
16x
16x
12x
12hrs
24hrs
36hrs
Hours post-transfection

Figure 29

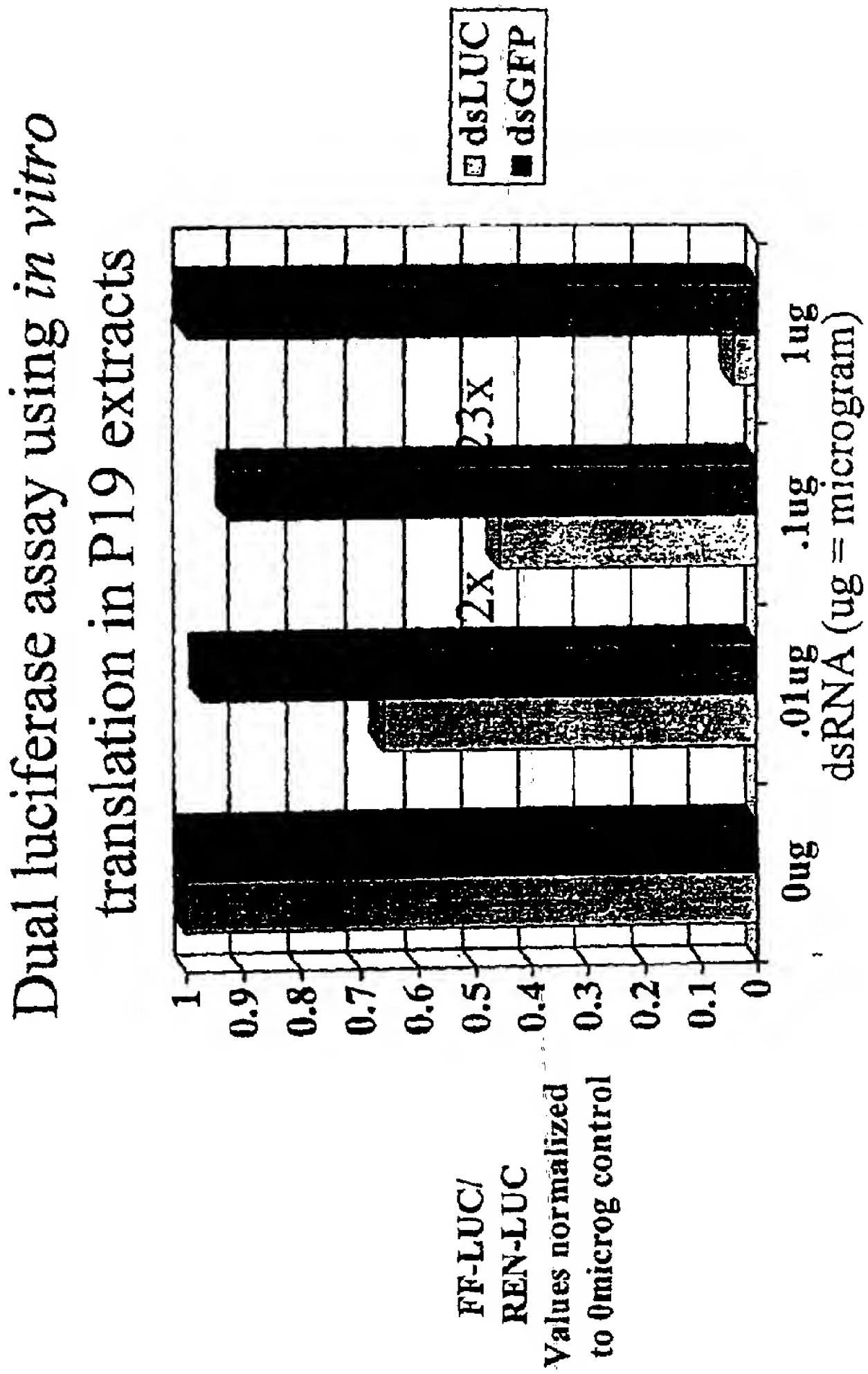

Dual luciferase assay using in vitro translation in P19 extracts
dsLUC
dsGFP
23X
2X
1
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
0ug
.01ug
.1ug
1ug
dsRNA (ug = microgram)
FF-LUC/ REN-LUC
Values normalized to 0microg control

Figure 30

Suppression of luciferase activity is dsRNA-specific for *in vitro* translation assay

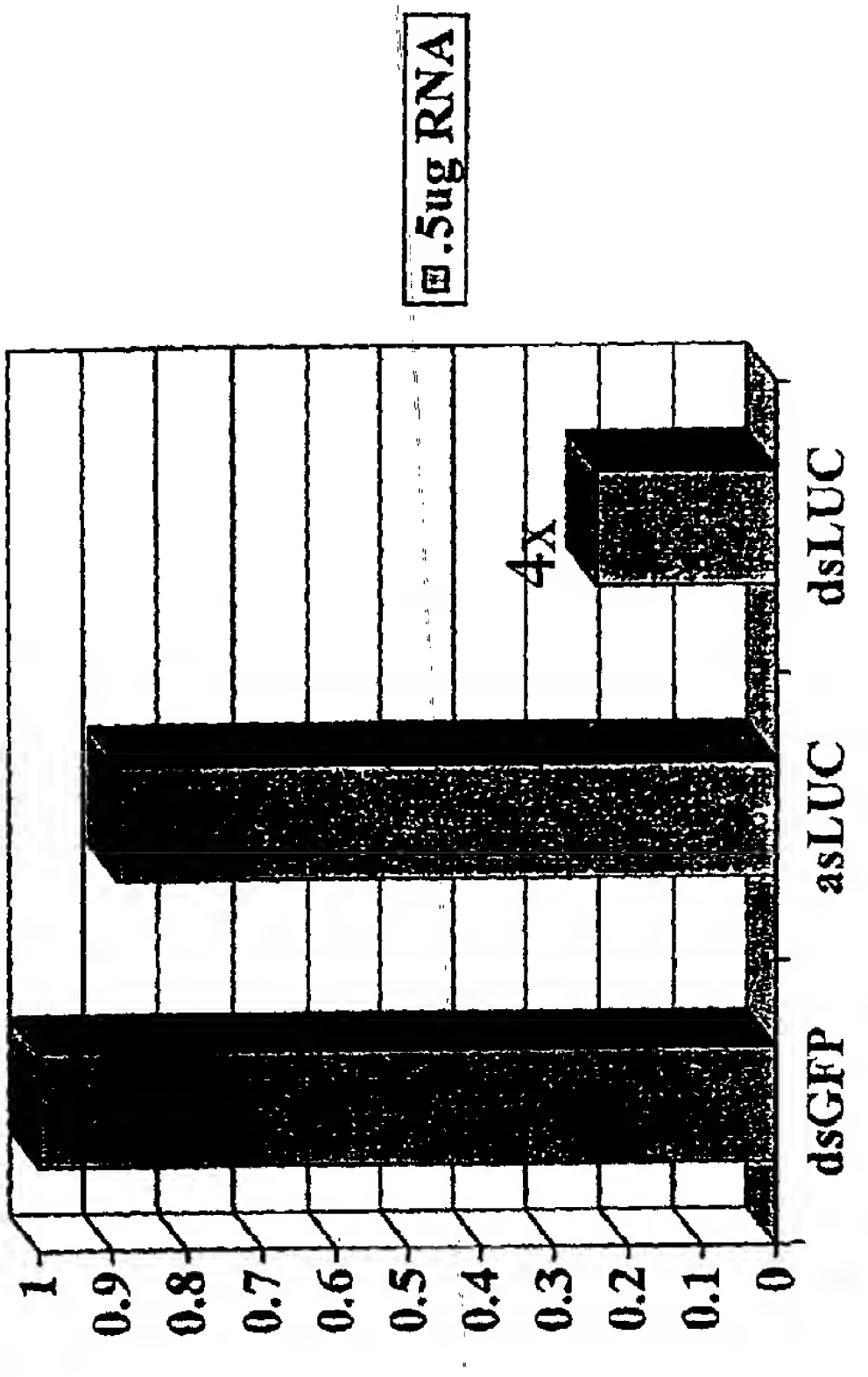


Figure 31

RNA molecules could specifically silence gene expression in invertebrate cells. Fire referred generally to this cellular process as RNA interference, or RNAi.

21. Fire received U.S. Patent No. 6,506,559 for his RNAi technology, which issued on January 14, 2003 (the "Fire Patent"). The essentially identical written disclosures of the Fire application and the Fire Patent are referred to collectively hereinafter as the "Fire Specification."

Facts Relating to Malpractice

22. From in or around 2001 until late 2008, R&G acted as principal outside patent prosecution counsel for CSHL.

23. Vincent was the R&G attorney primarily involved in the drafting and prosecution of the all of the Hannon Applications, as well as the '739 application and the '097 application, to which certain of the Hannon Applications claim priority. To date, CSHL has paid R&G approximately $420,000 in legal fees and disbursements that R&G has billed for its prosecution of the patent applications related to Dr. Hannon's shRNA technologies, and approximately $1,400,000 in fees and disbursements that R&G has billed for its prosecution of other applications.

24. The '097 application includes about 11 pages of text that Vincent copied essentially verbatim, without citation or attribution, from the published Fire application. Attached as Exhibit A is a "marked-up" version of the '097 application, in which text that is the same as text in the Fire Specification is highlighted. Vincent specifically carried over at least some portion of the copied Fire text found in the '097 application into all of the subsequently filed Hannon Applications.

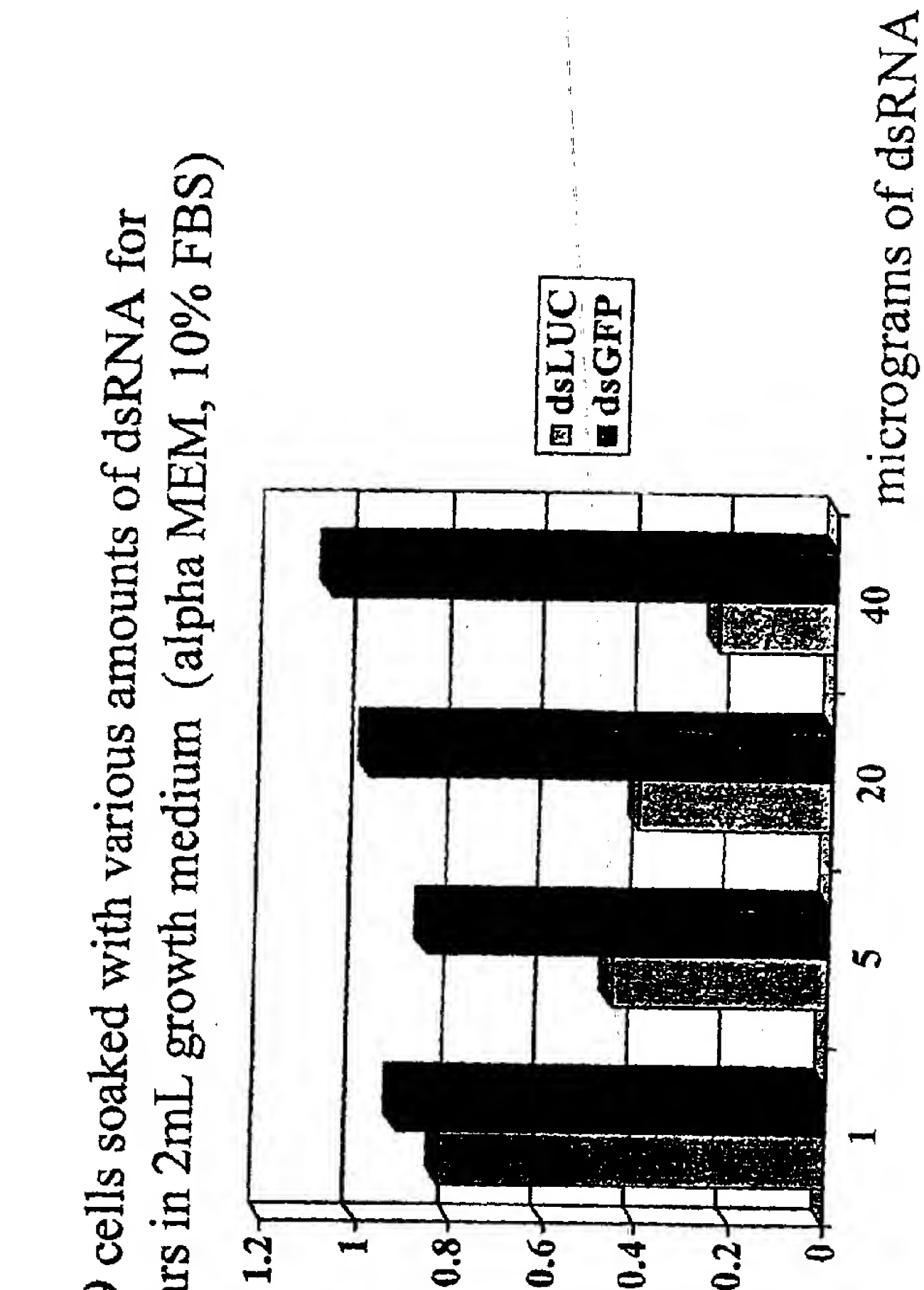
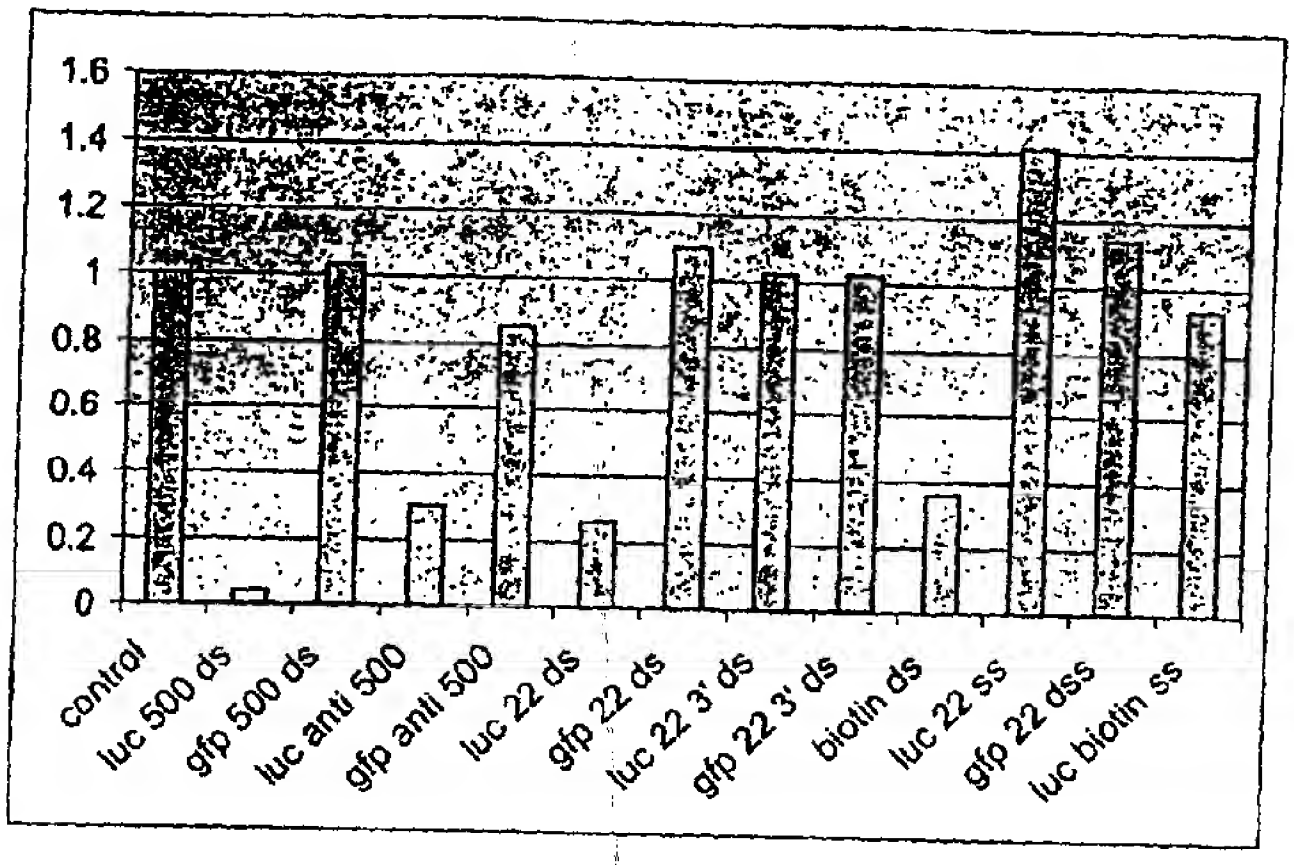

P19 cells soaked with various amounts of dsRNA for
12hrs in 2mL growth medium (alpha MEM, 10% FBS)
dsLUC
dsGFP
FF-LUC/
REN-LUC
Values normalized
to 0 microg control
1.2
1
0.8
0.6
0.4
0.2
0
1
5
20
40
micrograms of dsRNA

Figure 32

Figure 33


1.6
1.4
1.2
1
0.8
0.6
0.4
0.2
0
control
luc 500 ds
gfp 500 ds
luc anti 500
gfp anti 500
luc 22 ds
gfp 22 ds
luc 22 3' ds
gfp 22 3' ds
biotin ds
luc 22 ss
gfp 22 dss
luc biotin ss

METHODS AND COMPOSITIONS FOR RNA INTERFERENCE

RELATED APPLICATIONS

[0001] This application is a continuation-in-part of PCT application PCT/US01/08435, filed Mar. 16, 2001, and claims the benefit of U.S. Provisional applications U.S. Ser. No. 60/189,739 filed Mar. 16, 2000 and U.S. Ser. No. 60/243,097 filed Oct. 24, 2000. The specifications of such applications are incorporated by reference herein.

GOVERNMENT SUPPORT

[0002] Work described herein was supported by National Institutes of Health Grant R01-GM62534. The United States Government may have certain rights in the invention.

BACKGROUND OF THE INVENTION

[0003] "RNA interference", "post-transcriptional gene silencing", "quelling"—these different names describe similar effects that result from the overexpression or misexpression of transgenes, or from the deliberate introduction of double-stranded RNA into cells (reviewed in Fire A (1999) *Trends Genet* 15:358-363; Sharp PA (1999) *Genes Dev* 13:139-141; Hunter C (1999) *Curr Biol* 9:R440-R442; Baulcombe DC (1999) *Curr Biol* 9:R599-R601; Vaucheret et al. (1998) *Plant J* 16:651-659). The injection of double-stranded RNA into the nematode *Caenorhabditis elegans*, for example, acts systemically to cause the post-transcriptional depletion of the homologous endogenous RNA (Fire et al. (1998) *Nature* 391: 806-811; and Montgomery et al. (1998) *PNAS* 95:15502-15507). RNA interference, commonly referred to as RNAi, offers a way of specifically and potently inactivating a cloned gene, and is proving a powerful tool for investigating gene function. But the phenomenon is interesting in its own right; the mechanism has been rather mysterious, but recent research—the latest reported by Smardon et al. (2000) *Curr Biol* 10:169-178—is beginning to shed light on the nature and evolution of the biological processes that underlie RNAi.

[0004] RNAi was discovered when researchers attempting to use the antisense RNA approach to inactivate a *C. elegans* gene found that injection of sense-strand RNA was actually as effective as the antisense RNA at inhibiting gene function. Guo et al. (1995) *Cell* 81:611-620. Further investigation revealed that the active agent was modest amounts of double-stranded RNA that contaminate in vitro RNA preparations. Researchers quickly determined the 'rules' and effects of RNAi. Exon sequences are required, whereas introns and promoter sequences, while ineffective, do not appear to compromise RNAi (though there may be gene-specific exceptions to this rule). RNAi acts systemically—injection into one tissue inhibits gene function in cells throughout the animal. The results of a variety of experiments, in *C. elegans* and other organisms, indicate that RNAi acts to destabilize cellular RNA after RNA processing.

[0005] The potency of RNAi inspired Timmons and Fire (1998 *Nature* 395: 854) to do a simple experiment that produced an astonishing result. They fed to nematodes bacteria that had been engineered to express double-stranded RNA corresponding to the *C. elegans* unc-22 gene. Amazingly, these nematodes developed a phenotype similar to that of unc-22 mutants that was dependent on their food source. The ability to conditionally expose large numbers of nematodes to gene-specific double-stranded RNA formed the basis for a very powerful screen to select for RNAi-defective *C. elegans* mutants and then to identify the corresponding genes.

[0006] Double-stranded RNAs (dsRNAs) can provoke gene silencing in numerous in vivo contexts including Drosophila, *Caenorhabditis elegans*, planaria, hydra, trypanosomes, fungi and plants. However, the ability to recapitulate this phenomenon in higher eukaryotes, particularly mammalian cells, has not be accomplished in the art. Nor has the prior art demonstrated that this phenomena can be observe in cultured eukaryotes cells.

SUMMARY OF THE INVENTION

[0007] One aspect of the present invention provides a method for attenuating expression of a target gene in cultured cells, comprising introducing double stranded RNA (dsRNA) into the cells in an amount sufficient to attenuate expression of the target gene, wherein the dsRNA comprises a nucleotide sequence that hybridizes under stringent conditions to a nucleotide sequence of the target gene.

[0008] Another aspect of the present invention provides a method for attenuating expression of a target gene in a mammalian cell, comprising

[0009] (i) activating one or both of a Dicer activity or an Argonaut activity in the cell, and

[0010] (ii) introducing into the cell a double stranded RNA (dsRNA) in an amount sufficient to attenuate expression of the target gene, wherein the dsRNA comprises a nucleotide sequence that hybridizes under stringent conditions to a nucleotide sequence of the target gene.

[0011] In certain embodiments, the cell is suspended in culture; while in other embodiments the cell is in a whole animal, such as a non-human mammal.

[0012] In certain preferred embodiments, the cell is engineered with (i) a recombinant gene encoding a Dicer activity, (ii) a recombinant gene encoding an Argonaut activity, or (iii) both. For instance, the recombinant gene may encode, for a example, a protein which includes an amino acid sequence at least 50 percent identical to SEQ ID No. 2 or 4; or be defined by a coding sequence hybridizes under wash conditions of 2xSSC at 22° C. to SEQ ID No. 1 or 3. In certain embodiments, the recombinant gene may encode, for a example, a protein which includes an amino acid sequence at least 50 percent identical to the Argonaut sequence shown in FIG. 24.

[0013] In certain embodiments, rather than use a heterologous expression construct(s), an endogenous Dicer gene or Argonaut gene can be activated, e.g. by gene activation technology, expression of activated transcription factors or other signal transduction protein, which induces expression of the gene, or by treatment with an endogenous factor which upregualtes the level of expression of the protein or inhibits the degradation of the protein.

[0014] In certain preferred embodiments, the target gene is an endogenous gene of the cell. In other embodiments, the

target gene is an heterologous gene relative to the genome of the cell, such as a pathogen gene, e.g., a viral gene.

[0015] In certain embodiments, the cell is treated with an agent that inhibits protein kinase RNA-activated (PKR) apoptosis, such as by treatment with agents which inhibit expression of PKR, cause its destruction, and/or inhibit the kinase activity of PKF.

[0016] In certain preferred embodiments, the cell is a primate cell, such as a human cell.

[0017] In certain preferred embodiments, the length of the dsRNA is at least 20, 21 or 22 nucleotides in length, e.g., corresponding in size to RNA products produced by Dicer-dependent cleavage. In certain embodiments, the dsRNA construct is at least 25, 50, 100, 200, 300 or 400 bases. In certain embodiments, the dsRNA construct is 400-800 bases in length.

[0018] In certain preferred embodiments, expression of the target gene is attenuated by at least 5 fold, and more preferably at least 10, 20 or even 50 fold, e.g., relative to the untreated cell or a cell treated with a dsRNA construct which does not correspond to the target gene.

[0019] Yet another aspect of the present invention provides a method for attenuating expression of a target gene in cultured cells, comprising introducing an expression vector having a "coding sequence" which, when transcribed, produces double stranded RNA (dsRNA) the cell in an amount sufficient to attenuate expression of the target gene, wherein the dsRNA comprises a nucleotide sequence that hybridizes under stringent conditions to a nucleotide sequence of the target gene. An certain embodiments, the vector includes a single coding sequence for the dsRNA which is operably linked to (two) transcriptional regulatory sequences which cause transcription of in both directions (to form complementary transcripts of the coding sequence. In other embodiments, the vector includes two coding sequences which, respectively, give rise to the two complementary sequences which form the dsRNA when annealed. In certain embodiments, the vectors are episomal, e.g., and transfection is transient. In other embodiments, the vectors are chromosomally integrated, e.g., to produce a stably transfected cell line. Preferred vectors for forming such stable cell lines are the described in U.S. Pat. No. 6,025,192 and PCT publication WO/9812339, which are incorporated by reference herein.

[0020] Still another aspect of the present invention provides an assay for identifying nucleic acid sequences responsible for conferring a particular phenotype in a cell, comprising

[0021] (i) constructing a variegated library of nucleic acid sequences from a cell in an orientation relative to a promoter to produce double stranded DNA;

[0022] (ii) introducing the variegated dsRNA library into a culture of target cells, which cells have an activated Dicer activity or Argonaut activity;

[0023] (iii) identifying members of the library which confer a particular phenotype on the cell, and identifying the sequence from a cell which correspond, such as being identical or homologous, to the library member.

[0024] Yet another aspect of the present invention provides a method of conducting a drug discovery business comprising:

[0025] (i) identifying, by the subject assay, a target gene which provides a phenotypically desirable response when inhibited by RNAi;

[0026] (ii) identifying agents by their ability to inhibit expression of the target gene or the activity of an expression product of the target gene;

[0027] (iii) conducting therapeutic profiling of agents identified in step (b), or further analogs thereof, for efficacy and toxicity in animals; and

[0028] (iv) formulating a pharmaceutical preparation including one or more agents identified in step (iii) as having an acceptable therapeutic profile.

[0029] The method may include an additional step of establishing a distribution system for distributing the pharmaceutical preparation for sale, and may optionally include establishing a sales group for marketing the pharmaceutical preparation.

[0030] Another aspect of the present invention provides a method of conducting a target discovery business comprising:

[0031] (i) identifying, by the subject assay, a target gene which provides a phenotypically desirable response when inhibited by RNAi;

[0032] (ii) (optionally) conducting therapeutic profiling of the target gene for efficacy and toxicity in animals; and

[0033] (iii). licensing, to a third party, the rights for further drug development of inhibitors of the target gene.

[0034] Another aspect of the invention provides a method for inhibiting RNAi by inhibiting the expression or activity of an RNAi enzyme. Thus, the subject method may include inhibiting the acitivity of Dicer and/or the 22-mer RNA.

[0035] Still another aspect relates to the a method for altering the specificity of an RNAi by modifying the sequence of the RNA component of the RNAi enzyme.

[0036] Another aspect of the invention relates to purified or semi-purified preparations of the RNAi enzyme or components thereof. In certain embodiments, the preparations are used for identifying compounds, especially small organic molecules, which inhibit or potentiate the RNAi activity. Small molecule inhibitors, for example, can be used to inhibit dsRNA responses in cells which are purposefully being transfected with a virus which produces double stranded RNA.

[0037] The dsRNA construct may comprise one or more strands of polymerized ribonucleotide. It may include modifications to either the phosphate-sugar backbone or the nucleoside. The double-stranded structure may be formed by a single self-complementary RNA strand or two complementary RNA strands. RNA duplex formation may be initiated either inside or outside the cell. The dsRNA construct may be introduced in an amount which allows delivery of at least one copy per cell. Higher doses of double-stranded material may yield more effective inhibition. Inhibition is

sequence-specific in that nucleotide sequences corresponding to the duplex region of the RNA are targeted for genetic inhibition. dsRNA constructs containing a nucleotide sequences identical to a portion of the target gene is preferred for inhibition. RNA sequences with insertions, deletions, and single point mutations relative to the target sequence have also been found to be effective for inhibition. Thus, sequence identity may optimized by alignment algorithms known in the art and calculating the percent difference between the nucleotide sequences. Alternatively, the duplex region of the RNA may be defined functionally as a nucleotide sequence that is capable of hybridizing with a portion of the target gene transcript.

[0038] Yet another aspect of the invention pertains to transgenic non-human mammals which include a transgene encoding a dsRNA construct, preferably which is stably integrated into the genome of cells in which it occurs. The animals can be derived by oocyte microinjection, for example, in which case all of the nucleated cells of the animal will include the transgene, or can be derived using embryonic stem (ES) cells which have been transfected with the transgene, in which case the animal is a chimera and only a portion of its nucleated cells will include the transgene. In certain instances, the sequence-independent dsRNA response, e.g., the PKR response, is also inhibited in those cells including the transgene.

[0039] In still other embodiments, dsRNA itself can be introduced into an ES cell in order to effect gene silencing, and that phenotype will be carried for at least several rounds of division, e.g., into the progeny of that cell.

BRIEF DESCRIPTION OF THE DRAWINGS

[0040] **FIG. 1**: RNAi in S2 cells. a, Drosophila S2 cells were transfected with a plasmid that directs lacZ expression from the copia promoter in combination with dsRNAs corresponding to either human CD8 or lacZ, or with no dsRNA, as indicated. b, S2 cells were co-transfected with a plasmid that directs expression of a GFP-US9 fusion protein (12) and dsRNAs of either lacZ or cyclin E, as indicated. Upper panels show FACS profiles of the bulk population. Lower panels show FACS profiles from GFP-positive cells. c, Total RNA was extracted from cells transfected with lacZ, cyclin E, fizzy or cyclin A dsRNAs, as indicated. Northern blots were hybridized with sequences not present in the transfected dsRNAs.

[0041] **FIG. 2**: RNAi in vitro. a, Transcripts corresponding to either the first 600 nucleotides of Drosophila cyclin E (E600) or the first 800 nucleotides of lacZ (Z800) were incubated in lysates derived from cells that had been transfected with either lacZ or cyclin E (cycE) dsRNAs, as indicated. Time points were 0, 10, 20, 30, 40 and 60 min for cyclin E and 0, 10, 20, 30 and 60 min for lacZ. b, Transcripts were incubated in an extract of S2 cells that had been transfected with cyclin E dsRNA (cross-hatched box, below). Transcripts corresponded to the first 800 nucleotides of lacZ or the first 600, 300, 220 or 100 nucleotides of cyclin E, as indicated. Eout is a transcript derived from the portion of the cyclin E cDNA not contained within the transfected dsRNA. E-ds is identical to the dsRNA that had been transfected into S2 cells. Time points were 0 and 30 min. c, Synthetic transcripts complementary to the complete cyclin E cDNA (Eas) or the final 600 nucleotides (Eas600) or 300 nucleotides (Eas300) were incubated in extract for 0 or 30 min.

[0042] **FIG. 3**: Substrate requirements of the *RISC*. Extracts were prepared from cells transfected with cyclin E dsRNA. Aliquots were incubated for 30 min at 30° C. before the addition of either the cyclin E (E600) or lacZ (Z800) substrate. Individual 20-μl aliquots, as indicated, were pre-incubated with 1 mM $CaCl_2$ and 5 mM EGTA, 1 mM $CaCl_2$, 5 mM EGTA and 60 U of micrococcal nuclease, 1 mM $CaCl_2$ and 60 U of micrococcal nuclease or 10 U of DNase I (Promega) and 5 mM EGTA. After the 30-min pre-incubation, EGTA was added to those samples that lacked it. Yeast tRNA (1 μg) was added to all samples. Time points were at 0 and 30 min.

[0043] **FIG. 4**: The RISC contains a potential guide RNA. a, Northern blots of RNA from either a crude lysate or the S100 fraction (containing the soluble nuclease activity, see Methods) were hybridized to a riboprobe derived from the sense strand of the cyclin E mRNA. b, Soluble cyclin-E-specific nuclease activity was fractionated as described in Methods. Fractions from the anion-exchange resin were incubated with the lacZ, control substrate (upper panel) or the cyclin E substrate (centre panel). Lower panel, RNA from each fraction was analysed by northern blotting with a uniformly labelled transcript derived from sense strand of the cyclin E cDNA. *DNA* oligonucleotides were used as size markers.

[0044] **FIG. 5**: Generation of 22 mers and degradation of mRNA are carried out by distinct enzymatic complexes. A. Extracts prepared either from 0-12 hour Drosophila embryos or Drosophila S2 cells (see Methods) were incubated 0, 15, 30, or 60 minutes (left to right) with a uniformly-labeled double-stranded RNA corresponding to the first 500 nucleotides of the Drosophila cyclin E coding region. M indicates a marker prepared by in vitro transcription of a synthetic template. The template was designed to yield a 22 nucleotide transcript. The doublet most probably results from improper initiation at the +1 position. B. Whole-cell extracts were prepared from S2 cells that had been transfected with a dsRNA corresponding to the first 500 nt. of the luciferase coding region. S10 extracts were spun at 30,000×g for 20 minutes which represents our standard RISC extract[6]. S100 extracts were prepared by further centrifugation of S10 extracts for 60 minutes at 100,000×g. Assays for mRNA degradation were carried out as described previously[6] for 0, 30 or 60 minutes (left to right in each set) with either a single-stranded luciferase mRNA or a single-stranded cyclin E mRNA, as indicated. C. S10 or S100 extracts were incubated with cyclin E dsRNAs for 0, 60 or 120 minutes (L to R).

[0045] **FIG. 6**: Production of 22 mers by recombinant CG4792/Dicer. A. Drosophila S2 cells were transfected with plasmids that direct the expression of T7-epitope tagged versions of Drosha, CG4792/Dicer-1 and Homeless. Tagged proteins were purified from cell lysates by immunoprecipitation and were incubated with cyclin E dsRNA. For comparison, reactions were also performed in Drosophila embryo and S2 cell extracts. As a negative control, immunoprecipitates were prepared from cells transfected with a β-galactosidase expression vector. Pairs of lanes show reactions performed for 0 or 60 minutes. The synthetic marker (M) is as described in the legend to **FIG. 1**. B. Diagrammatic representations of the domain structures of CG4792/Dicer-1, Drosha and Homeless are shown. C. Immunoprecipitates were prepared from detergent lysates of S2 cells using an

antiserum raised against the C-terminal 8 amino acids of Drosophila Dicer-1 (CG4792). As controls, similar preparations were made with a pre-immune serum and with an immune serum that had been pre-incubated with an excess of antigenic peptide. Cleavage reactions in which each of these precipitates was incubated with an ~500 nt. fragment of Drosophila cyclin E are shown. For comparsion, an incubation of the substrate in Drosophila embryo extract was electrophoresed in parallel. D. Dicer immunoprecipitates were incubated with dsRNA substrates in the presence or absence of ATP. For comparison, the same substrate was incubated with S2 extracts that either contained added ATP or that were depleted of ATP using glucose and hexokinase (see methods). E. Drosophila S2 cells were transfected with uniformly, 32P-labelled dsRNA corresponding to the first 500 nt. of GFP. RISC complex was affinity purified using a histidine-tagged version of D.m. Ago-2, a recently identified component of the RISC complex (Hammond et al., in prep). RISC was isolated either under conditions in which it remains ribosome associated (ls, low salt) or under conditions that extract it from the ribosome in a soluble form (hs, high salt)[6]. For comparison, the spectrum of labelled RNAs in the total lysate is shown. F. Guide RNAs produced by incubation of dsRNA with a Dicer immunoprecipitate are compared to guide RNAs present in a affinity-purified RISC complex. These precisely comigrate on a gel that has single-nucleotide resolution. The lane labelled control is an affinity selection for RISC from cell that had been transfected with labeled dsRNA but not with the epitope-tagged D.m. Ago-2.

[0046] **FIG. 7**: Dicer participates in RNAi. A. Drosophila S2 cells were transfected with dsRNAs corresponding to the two Drosophila Dicers (CG4792 and CG6493) or with a control dsRNA corresponding to murine caspase 9. Cytoplasmic extracts of these cells were tested for Dicer activity. Transfection with Dicer dsRNA reduced activity in lysates by 7.4-fold. B. The Dicer-1 antiserum (CG4792) was used to prepare immunoprecipitates from S2 cells that had been treated as described above. Dicer dsRNA reduced the activity of Dicer-1 in this assay by 6.2-fold. C. Cells that had been transfected two days previously with either mouse caspase 9 dsRNA or with Dicer dsRNA were cotransfected with a GFP expression plasmid and either control, luciferase dsRNA or GFP dsRNA. Three independent experiments were quantified by FACS. A comparison of the relative percentage of GFP-positive cells is shown for control (GFP plasmid plus luciferase dsRNA) or silenced (GFP plamsid plus GFP dsRNA) populations in cells that had previously been transfected with either control (caspase 9) or Dicer dsRNAs.

[0047] **FIG. 8**: Dicer is an evolutionarily conserved ribonuclease. A. A model for production of 22 mers by Dicer. Based upon the proposed mechanism of action of Ribonuclease III, we propose that Dicer acts on its substrate as a dimer. The positioning of the two ribonuclease domains (RIIIa and RIIIb) within the enzyme would thus determine the size of the cleavage product. An equally plausible alternative model could be derived in which the RIIIa and RIIIb domains of each Dicer enzyme would cleave in concert at a single position. In this model, the size of the cleavage product would be determined by interaction between two neighboring Dicer enzymes. B. Comparison of the domain structures of potential Dicer homologs in various organisms (Drosophila—CG4792, CG6493, *C. elegans*—K12H4.8, Arabidopsis—CARPEL FACTORY[24], T25K16.4, AC012328_1, human Helicase-MOI[25] and *S. pombe*—YC9A_SCHPO). The ZAP domains were identified both by analysis of individual sequences with Pfam[27] and by Psi-blast[28] searches. The ZAP domain in the putative *S. pombe* Dicer is not detected by PFAM but is identified by Psi-Blast and is thus shown in a different color. For comparison, a domain structure of the RDE1/QDE2/ARGONAUTE family is shown. It should be noted that the ZAP domains are more similar within each of the Dicer and ARGONAUTE families than they are between the two groups. C. An alignment of the ZAP domains in selected Dicer and Argonaute family members is shown. The alignment was produced using ClustalW.

[0048] **FIG. 9**: Purification strategy for RISC. (second step in RNAi model).

[0049] **FIG. 10**: Fractionation of RISC activity over sizing column. Activity fractionates as 500 KD complex. Also, antibody to dm argonaute 2 cofractionates with activity.

[0050] FIGS. **11-13**: Fractionation of RISC over monoS, monoQ, Hydroxyapatite columns. Dm argonaute 2 protein also cofactionates.

[0051] **FIG. 14**: Alignment of dm argonaute 2 with other family members.

[0052] **FIG. 15**: Confirmation of dm argonaute 2. S2 cells were transfected with labeled dsRNA and His tagged argonaute. Argonaute was isolated on nickel agarose and RNA component was identified on 15% acrylamide gel.

[0053] **FIG. 16**: S2 cell and embryo extracts were assayed for 22 mer generating activity.

[0054] **FIG. 17**: RISC can be separated from 22 mer generating activity (dicer). Spinning extracts (S100) can clear RISC activity from supernatant (left panel) however, S100 spins still contain dicer activity (right panel).

[0055] **FIG. 18**: Dicer is specific for dsRNA and prefers longer substrates.

[0056] **FIG. 19**: Dicer was fractionated over several columns.

[0057] **FIG. 20**: Identification of dicer as enzyme which can process dsRNA into 22 mers. Various RNaseIII family members were expressed with n terminal tags, immunoprecipitated, and assayed for 22 mer generating activity (left panel). In right panel, antibodies to dicer could also precipitate 22 mer generating activity.

[0058] **FIG. 21**: Dicer requires ATP.

[0059] **FIG. 22**: Dicer produces RNAs that are the same size as RNAs present in RISC.

[0060] **FIG. 23**: Human dicer homolog when expressed and immunoprecipitated has 22 mer generating activity.

[0061] **FIG. 24**: Sequence of dm argonaute 2. Peptides identified by microsequencing are shown in underline.

[0062] **FIG. 25**: Molecular charaterization of dm argonaute 2. The presence of an intron in coding sequence was determined by northern blotting using intron probe. This results in a different 5' reading frame that that published genome seqeunce. Number of polyglutaine repeats was determined by genomic PCR.

[0063] FIG. 26: Dicer activity can be created in human cells by expression of human dicer gene. Host cell was 293. Crude extracts had dicer activity, while activity was absent from untransfected cells. Activity is not dissimilar to that seen in drosophila embryo extracts.

[0064] FIG. 27: An ~500 nt. fragment of the gene that is to be silenced (X) is inserted into the modified vector as a stable direct repeat using standard cloning procedures. Treatment with commercially available cre recombinase reverses sequences within the loxP sites (L) to create an inverted repeat. This can be stably maintained and amplified in an sbc mutant bacterial strain (DL759). Transcription in vivo from the promoter of choice (P) yields a hairpin RNA that causes silencing. A zeocin resistance marker is included to insure maintenance of the direct and inverted repeat structures; however this is non-essential in vivo and could be removed by pre-mRNA splicing if desired. Smith, N. A. et al. Total silencing by intron-spliced hairpin RNAs. *Nature* 407, 319-20 (2000).

[0065] FIG. 28: Hela, Chinese hamster ovary, and P19 (pluripotent, mouse embryonic carcinoma) cell lines transfected with plasmids expressing Photinus pyralis (firefly) Renilla reniformis (sea pansy) luciferases and with dsRNA 500 mers (400 ng), either homologous to firefly luciferase mRNA (dsLUC) or non-homologous (dsGFP). Dual luciferase assays were carried out using an Analytical Scientific Instruments model 3010 Luminometer. In this assay Renilla luciferase serves as an internal control for dsRNA-specific suppression of firefly luciferase activity. These data demonstrate that 500 mer dsRNA can specifically suppress cognate gene expression in vivo.

[0066] FIG. 29: P19 (a pluripontent, mouse embryonic cell line) cells transfected with plasmids expressing Photinus pyralis (firefly) Renilla reniformis (sea pansy) luciferases and with dsRNA 500 mers (500ng), either homologous to firefly luciferase mRNA (dsLUC) or non-homologous (dsGFP). Dual luciferase assays were carried out using an Analytical Scientific Instruments model 3010 Luminometer. In this assay Renilla luciferase serves as an internal control for dsRNA-specific suppression of firefly luciferase activity. These data further demonstrate that 500 mer dsRNA can specifically suppress cognate gene expression in vivo and that the effect is stable over time .

[0067] FIG. 30: S10 fractions from P19 cell lysates were used for in vitro translations of mRNA coding for Photinus pyralis (firefly) Renilla reniformis (sea pansy) luciferases. Translation reactions were programmed with various amounts of dsRNA 500 mers, either homologous to firefly luciferase mRNA (dsLUC) or non-homologous (dsGFP). Reactions were carried out at 30 degrees for 1 hour, after which dual luciferase assays were carried out using an Analytical Scientific Instruments model 3010 Luminometer. In this assay Renilla luciferase serves as an internal control for dsRNA-specific suppression of firefly luciferase activity. These data demonstrate that 500 mer dsRNA can specifically suppress cognate gene expression in vitro in a manner consistent with post-transcriptional gene silencing. Antisense firefly RNA did not differ significantly from dsGFP control (approximately 10%) (data not shown).

[0068] FIG. 31: S10 fractions from P19 cell lysates were used for in vitro translations of mRNA coding for Photinus pyralis (firefly) Renilla reniformis (sea pansy) luciferases. Translation reactions were programmed with dsRNA or asRNA 500 mers, either complementary to firefly luciferase mRNA (asLUC and dsLUC) or non-complementary (dsGFP). Reactions were carried out at 30 degrees for 1 hour, after a 30 min preincubation with dsRNA or asRNA. Dual luciferase assays were carried out using an Analytical Scientific Instruments model 3010 Luminometer. In this assay Renilla luciferase serves as an internal control for dsRNA-specific suppression of firefly luciferase activity. These data demonstrate that 500 mer double-stranded RNA (dsRNA) but not anti-sense RNA (asRNA) suppresses cognate gene expression in vitro in a manner consistent with post-transcriptional gene silencing.

[0069] FIG. 32: P19 cells were grown in 6-well tissue culture plates to approximately 60% confluence. Various amounts of dsRNA, either homologous to firefly luciferase mRNA (dsLUC) or non-homologous (dsGFP), were added to each well and incubated for 12 hrs under normal tissue culture conditions. Cells were then transfected with plasmids expressing Photinus pyralis (firefly) Renilla reniformis (sea pansy) luciferases and with dsRNA 500 mers (500 ng). Dual luciferase assays were carried out 12 hrs post-transfection using an Analytical Scientific Instruments model 3010 Luminometer. In this assay Renilla luciferase serves as an internal control for dsRNA-specific suppression of firefly luciferase activity. These data show that 500 mer dsRNA can specifically suppress cognate gene expression in vivo without transfection under normal tissue culture conditions.

[0070] FIG. 33: Is a graph illustrating the relative rate of expression luciferase in cells which are treated with various antisense and dsRNA constructs.

DETAILED DESCRIPTION OF THE CERTAIN PREFERRED EMBODIMENTS

I. Overview

[0071] The present invention provides methods for attenuating gene expression in a cell using gene-targeted double stranded RNA (dsRNA). The dsRNA contains a nucleotide sequence that hybridizes under physiologic conditions of the cell to the nucleotide sequence of at least a portion of the gene to be inhibited (the "target" gene).

[0072] A significant aspect to certain embodiments of the present invention relates to the demonstration in the present application that RNAi can in fact be accomplished in cultured cells, rather than whole organisms as described in the art.

[0073] Another salient feature of the present invention concerns the ability to carry out RNAi in higher eukaryotes, particularly in non-oocytic cells of mammals, e.g., cells from adult mammals as an example.

[0074] As described in further detail below, the present invention(s) are based on the discovery that the RNAi phenomenum is mediated by a set of enzyme activities, including an essential RNA component, that are evolutionarily conserved in eukaryotes ranging from plants to mammals.

[0075] One enzyme contains an essential RNA component. After partial purification, a multi-component nuclease (herein "RISC nuclease") co-fractionates with a discrete, 22-nucleotide RNA species which may confer specificity to

the nuclease through homology to the substrate mRNAs. The short RNA molecules are generated by a processing reaction from the longer input dsRNA. Without wishing to be bound by any particular theory, these 22 mer guide RNAs may serve as guide sequences that instruct the RISC nuclease to destroy specific mRNAs corresponding to the dsRNA sequences.

[0076] As illustrated in FIG. 33, double stranded forms of the 22-mer guide RNA can be sufficient in length to induce sequence-dependent dsRNA inhibition of gene expression. In the illustrated example, dsRNA contructs are administered to cells having a recombinant luciferase reporter gene. The control cell, e.g., no exogeneously added RNA, the level of expression of the luciferase reporter is normalized to be the value of "1". As illustrated, both long (500-mer) and short (22-mer) dsRNA constructs complementary to the luciferase gene could inhibit expression of that gene product relative to the control cell. On the other hand, similarly sized dsRNA complementary to the coding sequence for another protein, green fluorescence protein (GFP), did not significantly effect the expression of luciferase—indicating that the inhibitory phenomena was in each case sequence-dependent. Likewise, single stranded 22-mers of luciferase did not inhibit expression of that gene—indicating that the inhibitory phenomena is double stranded-dependent.

[0077] The appended examples also identify an enzyme, Dicer, that can produce the putative guide RNAs. Dicer is a member of the RNAse III family of nucleases that specifically cleave dsRNA and is evolutionarily conserved in worms, flies, plants, fungi and, as described herein, mammals. The enzyme has a distinctive structure which includes a helicase domain and dual RNAse III motifs. Dicer also contains a region of homology to the RDE1/QDE2/ARGONAUTE family, which have been genetically linked to RNAi in lower eukaryotes. Indeed, activation of, or overexpression of Dicer may be sufficient in many cases to permit RNA interference in otherwise non-receptive cells, such as cultured eukaryotic cells, or mammalian (non-oocytic) cells in culture or in whole organisms.

[0078] In certain embodiments, the cells can be treated with an agent(s) that inhibits the general double-stranded RNA response(s) by the host cells, such as may give rise to sequence-independent apoptosis. For instance, the cells can be treated with agents that inhibit the dsRNA-dependent protein kinase known as PKR (protein kinase RNA-activated). Double stranded RNAs in mammalian cells typically activate protein kinase PKR and leads to apoptosis. The mechanism of action of PKR includes phosphorylation and inactivation eIF2a (Fire (1999) *Trends Genet* 15:358). It has also been reported that induction of NF-κB by PKR is involved in apoptosis commitment and this process is mediated through activation of the IKK complex. This sequence-independent response may reflect a form of primitive immune response, since the presence of dsRNA is a common feature of many viral lifecycles.

[0079] As described herein, Applicants have demonstrated that the PKR response can be overcome in favor of the sequence-specific RNAi response. However, in certain instances, it can be desirable to treat the cells with agents which inhibit expression of PKR, cause its destruction, and/or inhibit the kinase activity of PKF are specifically contemplated for use in the present method. Likewise, overexpression of or agents which ectopic activate IF2αa. can be used. Other agents which can be used to suppress the PKR response include inhibitors of IKK phosphorylation of IκB, inhibitors of IκB ubiquitination, inhibitors of IκB degradation, inhibitors of NF-κB nuclear translocation, and inhibitors of NF-κB interaction with κB response elements.

[0080] Other inhibitors of sequence-independent dsRNA response in cells include the gene product of the vaccinia virus E3L. The E3L gene product contains two distinct domains. A conserved carboxy-terminal domain has been shown to bind double-stranded RNA (dsRNA) and inhibit the antiviral dsRNA response by cells. Expression of at least that portion of the E3L gene in the host cell, or the use of polypeptide or peptidomimetics thereof, can be used to suppress the general dsRNA response. Caspase inhibitors sensitized cells to killing by double-stranded RNA. Accordingly, ectopic expression or activated of caspases in the host cell can be used to suppress the general dsRNA response.

[0081] In other embodiments, the subject method is carried out in cells which have little or no general response to double stranded RNA, e.g., have no PKR-dependent dsRNA response, at least under the culture conditions. As illustrated in FIGS. 28-32, CHO and P19 cells can be used without having to inhibit PKR or other general dsRNA responses.

[0082] Thus, the present invention provides a process and compositions for inhibiting expression of a target gene in a cell, expecially a mammalian cell. In certain embodiments, the process comprises introduction of RNA (the "dsRNA construct") with partial or fully double-stranded character into the cell or into the extracellular environment. Inhibition is specific in that a nucleotide sequence from a portion of the target gene is chosen to produce the dsRNA construct. In preferred embodiments, the method utilizes a cell in which Dicer and/or Argonaute activities are recombinantly expressed or otherwise ectopically activated. This process can be (1) effective in attenuating gene expression, (2) specific to the targeted gene, and (3) general in allowing inhibition of many different types of target gene.

II. Definitions

[0083] For convenience, certain terms employed in the specification, examples, and appended claims are collected here."

[0084] As used herein, the term "vector" refers to a nucleic acid molecule capable of transporting another nucleic acid to that it has been linked. One type of vector is a genomic integrated vector, or "integrated vector", which can become integrated into the chromsomal DNA of the host cell. Another type of vector is an episomal vector, i.e., a nucleic acid capable of extra-chromosomal replication. Vectors capable of directing the expression of genes to that they are operatively linked are referred to herein as "expression vectors". In the present specification, "plasmid" and "vector" are used interchangeably unless otherwise clear from the context.

[0085] As used herein, the term "nucleic acid" refers to polynucleotides such as deoxyribonucleic acid (DNA), and, where appropriate, ribonucleic acid (RNA). The term should also be understood to include, as applicable to the embodiment being described, single-stranded (such as sense or antisense) and double-stranded polynucleotides.

[0086] As used herein, the term "gene" or "recombinant gene" refers to a nucleic acid comprising an open reading frame encoding a polypeptide of the present invention, including both exon and (optionally) intron sequences. A "recombinant gene" refers to nucleic acid encoding such regulatory polypeptides, that may optionally include intron sequences that are derived from chromosomal DNA. The term "intron" refers to a DNA sequence present in a given gene that is not translated into protein and is generally found between exons. As used herein, the term "transfection" means the introduction of a nucleic acid, e.g., an expression vector, into a recipient cell by nucleic acid-mediated gene transfer.

[0087] A "protein coding sequence" or a sequence that "encodes" a particular polypeptide or peptide, is a nucleic acid sequence that is transcribed (in the case of DNA) and is translated (in the case of mRNA) into a polypeptide in vitro or in vivo when placed under the control of appropriate regulatory sequences. The boundaries of the coding sequence are determined by a start codon at the 5' (amino) terminus and a translation stop codon at the 3' (carboxy) terminus. A coding sequence can include, but is not limited to, cDNA from procaryotic or eukaryotic mRNA, genomic DNA sequences from procaryotic or eukaryotic DNA, and even synthetic DNA sequences. A transcription termination sequence will usually be located 3' to the coding sequence.

[0088] Likewise, "encodes", unless evident from its context, will be meant to include DNA sequences that encode a polypeptide, as the term is typically used, as well as DNA sequences that are transcribed into inhibitory antisense molecules.

[0089] The term "loss-of-function", as it refers to genes inhibited by the subject RNAi method, refers a diminishment in the level of expression of a gene when compared to the level in the absense of dsRNA constructs.

[0090] The term "expression" with respect to a gene sequence refers to transcription of the gene and, as appropriate, translation of the resulting mRNA transcript to a protein. Thus, as will be clear from the context, expression of a protein coding sequence results from transcription and translation of the coding sequence.

[0091] "Cells,""host cells" or "recombinant host cells" are terms used interchangeably herein. It is understood that such terms refer not only to the particular subject cell but to the progeny or potential progeny of such a cell. Because certain modifications may occur in succeeding generations due to either mutation or environmental influences, such progeny may not, in fact, be identical to the parent cell, but are still included within the scope of the term as used herein.

[0092] The term "cultured cells" refers to cells suspended in culture, e.g., dispersed in culture or in the form tissue. It does not, however, include oocytes or whole embryos (including blastocysts and the like) which may be provided in culture. In certain embodiments, the cultured cells are adults cells, e.g., non-embryonic.

[0093] By "recombinant virus" is meant a virus that has been genetically altered, e.g., by the addition or insertion of a heterologous nucleic acid construct into the particle.

[0094] As used herein, the terms "transduction" and "transfection" are art recognized and mean the introduction of a nucleic acid, e.g., an expression vector, into a recipient cell by nucleic acid-mediated gene transfer. "Transformation", as used herein, refers to a process in which a cell's genotype is changed as a result of the cellular uptake of exogenous DNA or RNA, and, for example, the transformed cell expresses a dsRNA contruct.

[0095] "Transient transfection" refers to cases where exogenous DNA does not integrate into the genome of a transfected cell, e.g., where episomal DNA is transcribed into mRNA and translated into protein.

[0096] A cell has been "stably transfected" with a nucleic acid construct when the nucleic acid construct is capable of being inherited by daughter cells.

[0097] As used herein, a "reporter gene construct" is a nucleic acid that includes a "reporter gene" operatively linked to at least one transcriptional regulatory sequence. Transcription of the reporter gene is controlled by these sequences to which they are linked. The activity of at least one or more of these control sequences can be directly or indirectly regulated by the target receptor protein. Exemplary transcriptional control sequences are promoter sequences. A reporter gene is meant to include a promoter-reporter gene construct that is heterologously expressed in a cell.

[0098] As used herein, "transformed cells" refers to cells that have spontaneously converted to a state of unrestrained growth, i.e., they have acquired the ability to grow through an indefinite number of divisions in culture. Transformed cells may be characterized by such terms as neoplastic, anaplastic and/or hyperplastic, with respect to their loss of growth control. For purposes of this invention, the terms "transformed phenotype of malignant mammalian cells" and "transformed phenotype " are intended to encompass, but not be limited to, any of the following phenotypic traits associated with cellular transformation of mammalian cells: immortalization, morphological or growth transformation, and tumorigenicity, as detected by prolonged growth in cell culture, growth in semi-solid media, or tumorigenic growth in immuno-incompetent or syngeneic animals.

[0099] As used herein, "proliferating" and "proliferation" refer to cells undergoing mitosis.

[0100] As used herein, "immortalized cells" refers to cells that have been altered via chemical, genetic, and/or recombinant means such that the cells have the ability to grow through an indefinite number of divisions in culture.

[0101] The "growth state" of a cell refers to the rate of proliferation of the cell and the state of differentiation of the cell.

III. Exemplary Embodiments of Isolation Method

[0102] One aspect of the invention provides a method for potentiating RNAi by induction or ectopic activation of an RNAi enzyme in a cell (in vivo or in vitro) or cell-free mixtures. In preferred embodiments, the RNAi activity is activated or added to a mammalian cell, e.g., a human cell, which cell may be provided in vitro or as part of a whole organism. In other embodiments, the subject method is carried out using eukaryotic cells generally (except for oocytes) in culture. For instance, the Dicer enzyme may be activated by virtue of being recombinantly expressed or it

may be activated by use of an agent which (i) induces expression of the endogenous gene, (ii) stabilizes the protein from degradation, and/or (iii) allosterically modies the enzyme to increase its activity (by altering its Kcat, Km or both).

[0103] A. Dicer and Argonaut Activities

[0104] In certain embodiment, at least one of the activated RNAi enzymes is Dicer, or a homolog thereof. In certain preferred embodiments, the present method provides for ectopic activation of Dicer. As used herein, the term "Dicer" refers to a protein which (a) mediates an RNAi response and (b) has an amino acid sequence at least 50 percent identical, and more preferablty at least 75, 85, 90 or 95 percent identical to SEQ ID No. 2 or 4, and/or which can be encoded by a nucleic acid which hybridizes under wash conditions of 2×SSC at 22° C., and more preferably 0.2×SSC at 65° C., to a nucleotide represented by SEQ ID No. 1 or 3. Accordingly, the method may comprise introducing a dsRNA contruct into a cell in which Dicer has been recombinantly expressed or otherwise ectopically activated.

[0105] In certain embodiment, at least one of the activated RNAi enzymes is Argonaut, or a homolog thereof. In certain preferred embodiments, the present method provides for ectopic activation of Argonaut. As used herein, the term "Argonaut" refers to a protein which (a) mediates an RNAi response and (b) has an amino acid sequence at least 50 percent identical, and more preferablty at least 75, 85, 90 or 95 percent identical to the amino acid sequence shown in **FIG. 24**. Accordingly, the method may comprise introducing a dsRNA contruct into a cell in which Argonaut has been recombinantly expressed or otherwise ectopically activated.

[0106] This invention also provides expression vectors containing a nucleic acid encoding a Dicer or Argonaut polypeptides, operably linked to at least one transcriptional regulatory sequence. Operably linked is intended to mean that the nucleotide sequence is linked to a regulatory sequence in a manner which allows expression of the nucleotide sequence. Regulatory sequences are art-recognized and are selected to direct expression of the subject Dicer or Argonaut proteins. Accordingly, the term transcriptional regulatory sequence includes promoters, enhancers and other expression control elements. Such regulatory sequences are described in Goeddel; Gene Expression Technology: Methods in Enzymology 185, Academic Press, San Diego, Calif. (1990). For instance, any of a wide variety of expression control sequences, sequences that control the expression of a DNA sequence when operatively linked to it, may be used in these vectors to express DNA sequences encoding Dicer or Argonaut polypeptides of this invention. Such useful expression control sequences, include, for example, a viral LTR, such as the LTR of the Moloney murine leukemia virus, the early and late promoters of SV40, adenovirus or cytomegalovirus immediate early promoter, the lac system, the trp system, the TAC or TRC system, T7 promoter whose expression is directed by T7 RNA polymerase, the major operator and promoter regions of phage λ, the control regions for fd coat protein, the promoter for 3-phosphoglycerate kinase or other glycolytic enzymes, the promoters of acid phosphatase, e.g., Pho5, the promoters of the yeast α-mating factors, the polyhedron promoter of the baculovirus system and other sequences known to control the expression of genes of prokaryotic or eukaryotic cells or their viruses, and various combinations thereof. It should be understood that the design of the expression vector may depend on such factors as the choice of the host cell to be transformed and/or the type of protein desired to be expressed.

[0107] Moreover, the vector's copy number, the ability to control that copy number and the expression of any other proteins encoded by the vector, such as antibiotic markers, should also be considered.

[0108] The recombinant Dicer or Argonaut genes can be produced by ligating nucleic acid encoding a Dicer or Argonaut polypeptide into a vector suitable for expression in either prokaryotic cells, eukaryotic cells, or both. Expression vectors for production of recombinant forms of the subject Dicer or Argonaut polypeptides include plasmids and other vectors. For instance, suitable vectors for the expression of a Dicer or Argonaut polypeptide include plasmids of the types: pBR322-derived plasmids, pEMBL-derived plasmids, pEX-derived plasmids, pBTac-derived plasmids and pUC-derived plasmids for expression in prokaryotic cells, such as *E. coli*.

[0109] A number of vectors exist for the expression of recombinant proteins in yeast. For instance, YEP24, YIP5, YEP51, YEP52, pYES2, and YRP17 are cloning and expression vehicles useful in the introduction of genetic constructs into *S. cerevisiae* (see, for example, Broach et al. (1983) in Experimental Manipulation of Gene Expression, ed. M. Inouye Academic Press, p. 83, incorporated by reference herein). These vectors can replicate in *E. coli* due the presence of the pBR322 ori, and in *S. cerevisiae* due to the replication determinant of the yeast 2 micron plasmid. In addition, drug resistance markers such as ampicillin can be used. In an illustrative embodiment, a Dicer or Argonaut polypeptide is produced recombinantly utilizing an expression vector generated by sub-cloning the coding sequence of a Dicer or Argonaut gene.

[0110] The preferred mammalian expression vectors contain both prokaryotic sequences, to facilitate the propagation of the vector in bacteria, and one or more eukaryotic transcription units that are expressed in eukaryotic cells. The pcDNAI/amp, pcDNAI/neo, pRc/CMV, pSV2gpt, pSV2neo, pSV2-dhfr, pTk2, pRSVneo, pMSG, pSVT7, pko-neo and pHyg derived vectors are examples of mammalian expression vectors suitable for transfection of eukaryotic cells. Some of these vectors are modified with sequences from bacterial plasmids, such as pBR322, to facilitate replication and drug resistance selection in both prokaryotic and eukaryotic cells. Alternatively, derivatives of viruses such as the bovine papillomavirus (BPV-1), or Epstein-Barr virus (pHEBo, pREP-derived and p205) can be used for transient expression of proteins in eukaryotic cells. The various methods employed in the preparation of the plasmids and transformation of host organisms are well known in the art. For other suitable expression systems for both prokaryotic and eukaryotic cells, as well as general recombinant procedures, see Molecular Cloning A Laboratory Manual, 2nd Ed., ed. by Sambrook, Fritsch and Maniatis (Cold Spring Harbor Laboratory Press: 1989) Chapters 16 and 17.

[0111] In yet another embodiment, the subject invention provides a "gene activation" construct which, by homologous recombination with a genomic DNA, alters the transcriptional regulatory sequences of an endogenous Dicer or